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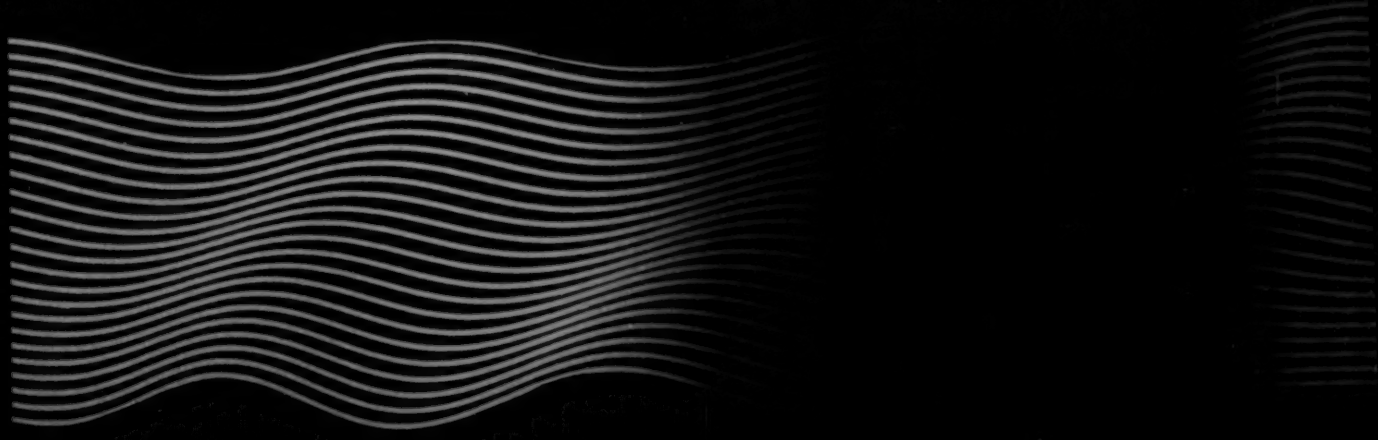

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REVIEW / SYNTHÈSE

Riverine salmonid egg burial depths: review of published data and implications for scour studies

Paul DeVries

Abstract: Published data on salmon, trout, and charr egg burial depths are highly variable and inconsistent. Primary sources of variation include elevation datum and portion of the egg pocket referenced to; differences in spawning behavior and the number, thickness, and location of egg pockets; relationships between egg depth, fish species, and corresponding size of female and spawning substrate and velocity characteristics; sampling method; presence of excavation barriers; redd superimposition; and scour and fill by hydraulic and other mechanical processes. Such sources of variability in the reported data have important implications for studies of scouring processes in salmonid spawning areas that require accurate identification of egg burial depths for predicting and preventing potential scour impacts. Cumulative measurement error and unexplained variation may amount to 5–20 cm or more in published values. The most relevant data for scour impact assessments are depths from the original stream bed elevation down to the top of the main egg pocket. Frequency distribution data are needed for determining probabilities and cumulative levels of scour impacts and for managing genetic diversity as well as population size. Preliminary depth threshold criteria are proposed for use now, pending further research.

Résumé : Les données publiées sur les profondeurs d'enfouissement des oeufs de saumon, de truite et d'omble sont hautement variables et manquent d'uniformité. Parmi les principales sources de variation figurent : l'élément de référence d'altitude et la partie de la chambre à oeufs dont on parle; des différences dans le comportement de frai et le nombre, l'épaisseur et l'emplacement des chambres d'oeufs; les relations entre la profondeur des oeufs, l'espèce de poisson et la taille correspondante de la femelle et le substrat du lieu de frai et les caractéristiques de vitesse; la méthode d'échantillonnage; la présence de barrières d'excavation; la superposition des nids de frai; et le creusement et le remblaiement par des moyens mécaniques hydrauliques et autres. Ces sources de variabilité dans les données publiées ont des répercussions importantes sur les études des processus de creusement dans les aires de frai de salmonidés qui nécessitent la détermination précise des profondeurs d'enfouissement des oeufs pour prévoir et prévenir les effets potentiels du creusement. L'erreur de mesures cumulée et la variation d'origine inexpliquée peuvent représenter 5–20 cm ou plus dans les valeurs publiées. Les données les plus pertinentes pour l'évaluation des effets du creusement sont les profondeurs depuis l'altitude initiale du lit du cours d'eau jusqu'à la partie supérieure de la chambre d'oeufs principale. Les données de distribution de fréquences sont nécessaires pour déterminer les probabilités et les degrés cumulés d'effet de creusement, ainsi que pour gérer la diversité génétique et la taille de la population. On propose des critères seuils préliminaires que l'on peut utiliser maintenant en attendant les résultats d'autres travaux de recherche.
[Traduit par la Rédaction]

Introduction

Salmon, trout, and charr spawning behavior is distinct from that of most other riverine fish species because of the manner in which eggs are deposited and incubated, the size of the eggs, and the length of the incubation period (Peterson and Quinn 1996). Male salmonids may participate in egg nest, or redd, construction (Crisp and Carling 1989) but characteristically only the female digs a functional redd in a gravel stream bed. The female releases her eggs into the depression where they are fertilized simultaneously by one or more males. She then covers the eggs with a layer of gravel that is relatively free of fine sediments. Usually, the female deposits the eggs in several pockets, laid in an upstream progression within a single general nest or redd (e.g., Hawke 1978). In contrast, most riverine species broadcast their eggs above the river bed, letting the eggs either settle to the bottom or be carried downstream in the current. Alternatively, they may produce small eggs that incubate over a relatively short time period (Scott and Crossman 1973). Salmonid eggs and embryos remain in the gravel for a relatively long time, ranging between roughly 2 and 8 months. The length of time between egg deposition and emergence depends on species, location, water temperature, dissolved oxygen levels, amount of infiltrated fine sediments, and other features that influence the rate of development or movement

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Paul DeVries. Department of Civil Engineering, P.O. Box 352700, University of Washington, Seattle, WA 98195, U.S.A. e-mail: pdevries@u.washington.edu

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within the gravel matrix (Bjornn and Reiser 1991; Groot and Margolis 1991).

The relatively long duration of the intragravel life stage implies that the survival of salmonid eggs and embryos is influenced more strongly by deposition and infiltration of fine sediments, changes in water quality, redd superimposition, disturbance by wading mammals, and stream bed scour and fill, than is the early life stage survival of other fish species. The depth to which the eggs are buried can affect the degree to which each of the above factors influences the survival to emergence. In the case of stream bed scour, this specific phase of the life cycle can limit the size of salmonid populations if the substrate is excavated down to, and (or) begins moving at, the elevation of the eggs (McNeil 1966; Seegrist and Gard 1972; Kondolf et al. 1991). The term "scour depth" is used here to refer to the difference in elevation (at a specific location in the channel) between the original stream bed surface at the time of spawning and the bottom of the active bedload transport layer during individual peak runoff events, including possible net excavation of local material.

Montgomery et al. (1996) studied scour depths in a small west-coast stream and determined that stream bed scour depths during frequent, bankfull flows were generally shallower than, or near the smaller values of, depths to the top of chum salmon (*Oncorhynchus keta*) egg pockets. They postulated that egg burial depth could be an evolutionary adaptation to scour events that occur on an episodic basis in coarse-bed channels. This observation may well be true for most other riverine salmonid populations and more work is needed on the subject. A literature search of the data on egg burial depth was conducted to evaluate further the hypothesized relationship between scour depth and the incubation success of different riverine salmonid species. Such data have not been summarized within a consistent, comprehensive source to date. This paper (*i*) summarizes egg burial depth information in a form useful to fisheries and environmental professionals interested in assessing scour-related impacts of land-use activities, (*ii*) identifies potential sources of variation, (*iii*) evaluates the utility of existing data for scour studies, and (*iv*) proposes threshold criteria pending collection of new data.

Egg depth data

Data on egg burial depths for riverine salmon, trout, and charr species vary widely (Table 1). Species for which data were found included chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), chum salmon, pink salmon (*O. gorbuscha*), Atlantic salmon (*Salmo salar*), (non-anadromous) kokanee and (anadromous) sockeye salmon (*O. nerka*), (non-anadromous) rainbow and (anadromous) steelhead trout (*O. mykiss*), resident cutthroat trout (*O. clarki*), (non-anadromous) brown and (anadromous) sea trout (*S. trutta*), and golden trout (*O. aguabonita*). Data were also compiled for charr species including brook trout (*Salvelinus fontinalis*), bull trout (*S. confluentus*), and Dolly Varden trout (*S. malma*). The compilation was intended to be as exhaustive as possible. There undoubtedly are other data available in less accessible, diverse sources such as university and fishery agency project reports that were not identified in the current literature search. However, the collection here represents a significant amount of the best information available in the refereed and nonrefereed literature and may be considered descriptive of individual species' egg-laying behavior.

Authors have not determined or reported egg burial depths consistently. They have reported depths from two different types of elevation datum: the level of the original undisturbed substrate and the level of the top of the redistributed, overlying gravel (Fig. 1). It is not always clear which datum applies. I present my own best estimate where possible in Table 1 when the respective publication did not specify the datum clearly but implied it in the text. Data have furthermore been reported for either the depth down to the top, center, or bottom of the egg pocket (Fig. 1), or for individual eggs throughout the thickness of the pocket. Like the datum, it was not always reported explicitly and interpretation was occasionally necessary in the preparation of Table 1. Redd excavation depths were assumed to be analogous to the top of the egg pocket: the eggs settle among the crevices of the redd bottom during the spawning act. Redd pit depth data (postspawning) were not included here because they may underestimate egg depths: the female is digging only deep enough to cover her last eggs.

The variable, nonstandard formats used to report egg burial depths made it impossible to analyze the data using exploratory statistics as Kondolf and Wolman (1993) did for evaluating substrate size characteristics selected by spawning salmonids. Many data were reported as ranges only, limiting their usefulness for frequency-based analyses. Instead, I created charts to depict the range of the data and to facilitate development of first-order depth criteria for assessment of scour risks (Fig. 2). The charts were based only on measured data for which the distance down to the top or bottom of the pocket, and the corresponding reference datum, could be determined reasonably. The smaller value of each reported range of depths of discrete eggs in Table 1 was assumed analogous to the top, and the larger value to the bottom of the egg pocket.

Larger species can clearly bury their eggs at greater depths than smaller species (Fig. 2). The listed order of species in Fig. 2 was based on general species size differences, from largest (chinook salmon) to smallest (brook trout and kokanee salmon). Although the trend is less clear for the shallowest depths, the smaller species appear to bury their eggs closer to the stream bed surface than do the larger species. A few species seem to dig less deeply than might be expected on the basis of size considerations alone (e.g., steelhead trout and Atlantic salmon), whereas others appear to dig more deeply (e.g., pink salmon). This may be an artifact of the small size of the data base, a biologic response to hydrologic and geomorphic features, or may be due to several sources of variation that are described in the next section.

Sources of variability, and their implications for scour assessments

The wide range found in egg burial depth values implies that there is presently considerable uncertainty inherent in scour assessments designed to relate anthropogenic changes in sediment inputs and flood hydrology to the survival of the salmonid incubation life stage (e.g., Schuett-Hames et al. 1996). Accurate knowledge of egg burial depths allows identification of the elevation at which scouring impacts can be expected, either because of lowering of the stream bed elevation caused by sediment transport imbalances or as a result of mechanical

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Table 1. Summary of reported egg burial depth data, to nearest centimetre.

Species/authors	Datum[a]	Portion of pocket[a]	Depth (cm) Mean	*n*	Range	Location	Method	Comments[b]
Atlantic salmon								
Belding (1934)	Original level	Top			15–30	Canada	Observation	Depth of pit prior to egg deposition
White (1942)	Original level	Top			15–30+	Nova Scotia	Observation	Depth of pit prior to egg deposition
Ottaway et al. (1981)	Overlying gravel	Bottom	18	1		United Kingdom	Freeze	Main egg pocket; RFL = 67 cm
Barlaup et al. (1994)	Overlying gravel	Bottom	27	10		Norway	Excavation	1 SD = 3.9 cm
Heggberget et al. (1988)	Overlying gravel	Center	18	159		Norway	Excavation	1 SD = 6.0 cm
Ottaway et al. (1981)	Overlying gravel	Discrete eggs		1	10–18	United Kingdom	Freeze	RFL = 67 cm
Crisp and Carling (1989)	Overlying gravel	Discrete eggs	17–23	3		United Kingdom	Freeze	Means of redds with >4 eggs; RFL = 51–85 cm
Brook trout								
Needham (1961)	Original level	Top		1	10–15	California	Observation	Depth of pit prior to egg deposition
Reiser and Wesche (1977)	Original level	Top			<9	Wyoming	Excavation	Pit depths; RFL <26 cm
Young et al. (1989)	Overlying gravel	Bottom	8	31	6–12	Wyoming	Freeze	1 Standard Error = 1.7 cm; RFL = 15–30 cm
Witzel and MacCrimmon (1983)	Overlying gravel	Discrete eggs			<15	Ontario	McNeil	Depths noted to rarely exceed this
Brown trout								
Hobbs (1937)	Original level	Bottom			15–25	New Zealand	Excavation	
Hobbs (1940)	Original level	Discrete eggs			20–25	New Zealand		Usual depth
Hobbs (1937)	Original level	Top	20			New Zealand	Excavation	Usual depth
Jones and Ball (1954)	Original level	Top	8			United Kingdom	Observation	"Typical" trout redd
Jones and Ball (1954)	Original level	Top		4	6–10	United Kingdom	Observation	Approximate depths of egg pockets
Reiser and Wesche (1977)	Original level	Top			<17	Wyoming	Excavation	Pit depths; RFL <41 cm
Ottaway et al. (1981)	Overlying gravel	Bottom	9	5	7–14	United Kingdom	Freeze	Main egg pocket; RFL = 26–35 cm
Grost et al. (1991)	Overlying gravel	Bottom	12	75	2–23	Wyoming	Freeze	RFL = 20–50 cm
Heggberget et al. (1988)	Overlying gravel	Center	12	73		Norway	Excavation	1 SD = 12 cm
Reiser and Wesche (1977)	Overlying gravel	Discrete eggs	9–12			Wyoming	Excavation	Normal depths of egg pockets; RFL <41 cm
Ottaway et al. (1981)	Overlying gravel	Discrete eggs		5	0–25	United Kingdom	Freeze	RFL = 26–35 cm
Witzel and MacCrimmon (1983)	Overlying gravel	Discrete eggs			>13	Ontario	McNeil	Specified as general burial depth
Elliott (1984)	Overlying gravel	Discrete eggs	4	16	2–12	United Kingdom	Excavation	Mean is for modal depths; RFL = 18–28 cm
Crisp and Carling (1989)	Overlying gravel	Discrete eggs	7–16	6		United Kingdom	Freeze	Means of redds with >4 eggs; RFL = 24–44 cm
Grost et al. (1991)	Overlying gravel	Discrete eggs	11	75	2–20	Wyoming	Freeze	Discrete samples; mean egg depths; RFL = 20–50 cm
Grost et al. (1991)	Overlying gravel	Discrete eggs	12			Wyoming	Freeze	Samples with >19 eggs; RFL = 20–50 cm
Hardy (1963)	Overlying gravel	Top	16	8	10–20	New Zealand	Excavation	Stranded redds; redd means
Hardy (1963)	Overlying gravel	Top			8–22	New Zealand	Excavation	Stranded redds; all data
Grost et al. (1991)	Overlying gravel	Top	9	75	2–16	Wyoming	Freeze	RFL = 20–50 cm
Bull trout								
McPhail and Murray (1979)	Original level	Top			10–16	B.C.		Redd excavation depth
Leggett (1980)	Original level	Top			10–15	B.C.	Observation	Redd excavation depth; spawning in artificial channel
Block (1955)	Overlying gravel	Top	20	1		Montana		
Leggett (1980)	Overlying gravel	Top			15–20	B.C.	Excavation	Spawning in artificial channel

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Table 1 (*continued*).

Species/authors	Datum[a]	Portion of pocket[a]	Depth (cm) Mean	*n*	Range	Location	Method	Comments[b]
Shepard et al. (1984*a*)	Overlying gravel	Top			>14	Montana	McNeil	
Shepard et al. (1984*b*)	Overlying gravel	Top			10–20	Montana		
Heimer (1965)					8–15	Idaho		Cited in Shepard et al. 1984*b*
Allan (1980)					3–18	Alberta		Cited in Shepard et al. 1984*b*
Chinook salmon								
Miller (1985)	Original level	Bottom	30			Washington		General criterion based in part on own data
Hobbs (1937)	Original level	Discrete eggs			30–41	New Zealand	Excavation	Considered 99% of eggs to be within this layer
Vronskii and Leman (1991)	Original level	Discrete eggs			21–50	USSR		Depths at which eggs reportedly found most frequently
Hobbs (1937)	Original level	Top			15–46	New Zealand	Observation	Redd excavation depths
Hobbs (1937)	Original level	Top			>20	New Zealand	Excavation	Eggs usually expected below this depth
Burner (1951)	Original level	Top	22–27		5–51	Washington	Observation	Deepest part of redd measured at different time intervals
Briggs (1953)	Original level	Top		2	28–36	California	Observation	Depth of pit prior to egg deposition
Scott and Crossman (1973)	Original level	Top			<31	Canada		Redd excavation depth; general criterion
Miller (1985)	Original level	Top	15			Washington		General criterion based in part on own data
Vronskiy (1972)	Overlying gravel	Bottom	53	10	40–80	USSR	Excavation	Maximum depths in 10 mounds
Chapman et al. (1986)	Overlying gravel	Bottom	29	54	19–37	Columbia River	Probing	May be underestimates according to authors
Hawke (1978)	Overlying gravel	Center	36	7	32–41	New Zealand	Excavation	Stranded redds; redd means
Hawke (1978)	Overlying gravel	Center			18–43	New Zealand	Excavation	Stranded redds; all data
Briggs (1953)	Overlying gravel	Top	28	8	20–36	California	Excavation	
Vronskiy (1972)	Overlying gravel	Top	21	10	10–46	USSR	Excavation	Minimum depths in 10 mounds
Chapman et al. (1986)	Overlying gravel	Top	19	116	10–33	Columbia River	Excavation	Depth to first embryos encountered
Chum salmon								
Bruya (1981)	Original level	Bottom		4	20–40	Washington	Freeze	Gravel disturbance by spawners (control); RFL = 65–74 cm
Burner (1951)	Original level	Top	22		8–43	Washington	Observation	Deepest part of redd measured at different time intervals
Scott and Crossman (1973)	Original level	Top			<41	Washington		Redd excavation depth; general criterion
Salo (1991)	Original level	Top			20–40	North America		General criterion for redd pit depth prior to egg deposition
Montgomery et al. (1996)	Original level	Top	23	40	10–49	Washington	Excavation	
Bruya (1981)	Overlying gravel	Discrete eggs		4	10–30	Washington	Freeze	93% of eggs recovered (control); RFL = 65–74 cm
Tripp and Poulin (1986)	Overlying gravel	Discrete eggs		34	0–45	B.C.	Probing	
Tripp and Poulin (1986)	Overlying gravel	Discrete eggs			10–35	B.C.	Probing	Majority of eggs (>90%)
L. Powell (in Scrivener and Brownlee 1989)	Overlying gravel	Discrete eggs			5–20	B.C.	Freeze	Cited personal communication
K.V. Koski (in Scrivener and Brownlee 1989)		Discrete eggs			10–50	Washington		Cited personal communication
K.V. Koski (in Scrivener and Brownlee 1989)		Discrete eggs	22			Alaska		Cited personal communication
Bazarkin (1990)		Discrete eggs			30–40	USSR		
Meehan and Bjornn (1991)		Discrete eggs			15–30	North America		General criterion

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Table 1 (*continued*).

Species/authors	Datum[a]	Portion of pocket[a]	Depth (cm) Mean	*n*	Range	Location	Method	Comments[b]
Coho salmon								
Gribanov (1962)	Original level	Discrete eggs		4	10–15	USSR	Excavation	Opened 2m × 2m of level stream bed in mass spawning area
Burner (1951)	Original level	Top	20		8–51	Washington	Observation	Deepest part of redd measured at different time intervals
Briggs (1953)	Original level	Top		2	20–25	California	Observation	Depth of pit prior to egg deposition
van den Berghe and Gross (1984)	Original level	Top	15	13	9–27	Washington	Observation	Redd excavation depths; RFL = 47–74 cm
Zorbidi (1988)	Overlying gravel	Bottom	33	10	16–55	USSR	Excavation	
Briggs (1953)	Overlying gravel	Top	25	16	18–38	California	Excavation	
Gribanov (1962)	Overlying gravel	Top	22	15	15–27	USSR	Excavation	Examined during spawning season
Gribanov (1962)	Overlying gravel	Top	21	9	16–30	USSR	Excavation	Examined 2 months after spawning
Zorbidi (1988)	Overlying gravel	Top	12	10	6–20	USSR	Excavation	
Koski (1966)	Overlying gravel	Discrete eggs			18–28	Oregon	Excavation	Embryos prior to emergence in two redds
Tripp and Poulin (1986)	Overlying gravel	Discrete eggs		30	0–45	B.C.	Probing	
Tripp and Poulin (1986)	Overlying gravel	Discrete eggs			20–35	B.C.	Probing	Majority of eggs (>90%)
Cutthroat trout								
Smith (1941)	Original level	Top			10–13	California	Observation	Depth of pit prior to egg deposition
Smith (1941)	Overlying gravel	Top			15–20	California	Observation	Apparent depth of refilled gravel
Wydoski and Whitney (1979)	Overlying gravel	Top			13–18	Washington		General criterion
Kiefling (1978)	Overlying gravel	Top			15–20	Wyoming	Excavation	
Dolly Varden trout								
Blackett (1968)	Original level	Top			15–20	Alaska	Observation	Redd excavation depth
Scott and Crossman (1973)	Original level	Top			<31	Canada		Redd excavation depth; general criterion
Golden trout								
Knapp and Vredenburg (1996)	Original level	Bottom	5	65	4–6	California	Excavation	Sampled 29 redds
Kokanee salmon								
Scott and Crossman (1973)	Original level	Top			5–10	Canada		Redd excavation depth; general criterion
Pink salmon								
Scott and Crossman (1973)	Original level	Top			<46	Canada		Redd excavation depth; general criterion
Dvinin (1957, 1959)	Overlying gravel	Discrete eggs	15–25		7–45	USSR	Excavation	Eggs rarely found deeper than 30–35 cm; cited in Raleigh and Nelson 1985
Vasilenko-Lukina (1962)	Overlying gravel	Discrete eggs	25–30	29		USSR	Excavation	
Enyutina (1974)	Overlying gravel	Discrete eggs	20–30		18–50	USSR		
Rukhlov (1969)			32–23			USSR		Mean deposition depth changes over time due to scouring
Rainbow trout								
Hobbs (1937)	Original level	Top	20	4		New Zealand	Excavation	Approximate depth of egg pockets
Hooper (1973)	Original level	Top	15			California	Observation	Most excavation depths (out of 10 redds); RFL = 30–36 cm

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Table 1 (*concluded*).

Species/authors	Datum[a]	Portion of pocket[a]	Depth (cm) Mean	*n*	Range	Location	Method	Comments[b]
Sea (brown) trout								
Ottaway et al. (1981)	Overlying gravel	Bottom	21	2	20–22	United Kingdom	Freeze	Main egg pocket; RFL = 55–57 cm
Barlaup et al. (1994)	Overlying gravel	Bottom	17	10		Norway	Excavation	1 SD = 5.2 cm
Ottaway et al. (1981)	Overlying gravel	Discrete eggs		2	3–22	United Kingdom	Freeze	RFL = 55–57 cm
Elliott (1984)	Overlying gravel	Discrete eggs	17	22	5–24	United Kingdom	Excavation	Mean is for modal depths; RFL = 25–45 cm
Crisp and Carling (1989)	Overlying gravel	Discrete eggs	8–26	24		United Kingdom	Freeze	Means of redds with >4 eggs; RFL = 31–74 cm
Sockeye salmon								
Mathisen (1962)	Original level	Center		22	8–13	Alaska	Excavation	
Mathisen (1962)	Original level	Center		149	15–23	Alaska	Excavation	
Mathisen (1962)	Original level	Center		27	25–30	Alaska	Excavation	
Burner (1951)	Original level	Top	11–14		5–28	Washington	Observation	Deepest part of redd measured at different time intervals
Kuznetsov (1928)	Overlying gravel	Discrete eggs			9–29	USSR	Excavation?	Majority of eggs deeper than 17 cm; cited in Foerster 1968
Mathisen (1955)	Overlying gravel	Discrete eggs	19	130		Alaska	Excavation	Pockets with <11% of eggs dead (unfertilized)
Mathisen (1955)	Overlying gravel	Discrete eggs	18	28		Alaska	Excavation	Pockets with >89% of eggs dead (unfertilized)
Mathisen (1955)	Overlying gravel	Top			18–28	Alaska	Excavation	Pocket depths within single redd; RFL = 59 cm
Mathisen (1955)	Overlying gravel	Top	12	1		Alaska	Excavation	Diagram of pocket within single redd
Steelhead trout								
Miller (1985)	Original level	Bottom	30			Washington		General criterion based in part on own data
Needham and Taft (1934)	Original level	Top		1	10–13	California	Observation	Depth of pit prior to egg deposition
Briggs (1953)	Original level	Top	20	1		California	Observation	Depth of pit prior to egg deposition
Shapovolov and Taft (1954)	Original level	Top			10–30	California	Observation	Depth of pit prior to egg deposition
Miller (1985)	Original level	Top	15			Washington		General criterion based in part on own data
Briggs (1953)	Overlying gravel	Top	21	13	15–28	California	Excavation	
Wydoski and Whitney (1979)	Overlying gravel	Top			<31	Washington		General criterion

[a]See Fig. 1 for a depiction of relevant geometry.
[b]RFL = Range of female lengths.

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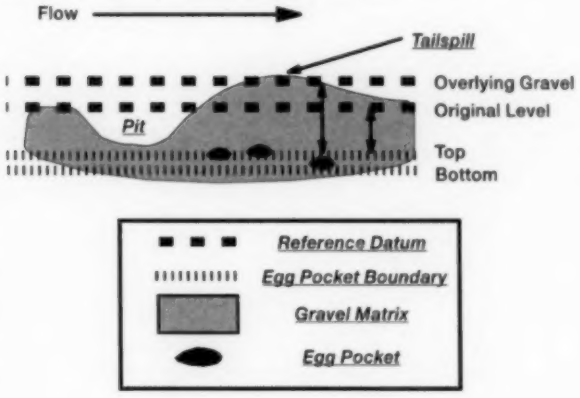
Fig. 1. Schematic of a generic salmonid redd depicting geometries applicable to scour studies. Depths to the top of an egg pocket are indicated for each reference datum. An egg pocket is considered to consist of a cluster of five or more eggs; individual eggs may be scattered outside of the main pocket(s) as well.



crushing and washing out of eggs and embryos during episodes of bedload transport. Predicted scour depths can then be related to egg burial depths to determine potential influences on salmonid populations. However, the magnitude of error in the reported data appears to be much larger than the resolution needed. Data collected by Montgomery et al. (1996) suggest that small increases in scour depth (on the order of several centimetres) may seriously reduce incubation survival. It is therefore important to identify and account for the potential sources of variability in reported egg depth data before evaluating measured or predicted scour depth increases.

I divided sources of variability into two general categories: variation linked more directly to (*i*) sampling considerations or (*ii*) fish excavation capability and spawning behavior. Each potential form of variation is identified and discussed below in the context of determining scour impacts to the incubation life-history stage. I subsequently estimated the maximum error magnitude that each source may contribute to a study of egg burial depths.

Sampling variability

Greatest sampling-related variability is introduced by the form of elevation datum used and referenced portion of the egg pocket. Choice of datum causes differences on the order of 0–5 cm. Differences of 5–10 cm may occur depending on which boundary of the egg pocket is referenced (Fig. 2). Differences in elevation between the first eggs encountered and the top of the main egg cluster likely introduce variation on the order of 1–2 cm.

The depth below the overlying gravel may not be a sufficiently consistent measure for scour studies because the difference between the tailspill mound and surrounding stream bed elevations varies with position on the redd. Also, the burial depth of egg pockets under the hump of the tailspill may become shallower over the course of the incubation period if the redd is leveled gradually by the stream flow. Stuart (1953) noted such a decrease in brown trout egg burial depths relative to the overlying gravel surface, on the order of half the original value. Crisp and Carling (1989) and others have also noted that redds will generally level out with sufficient flow. Scour depth predictions for spawning areas can be linked more accurately to the original elevation of the surrounding, undisturbed gravel surface than to the elevation of specific portions of the tailspill (Fig. 1) because most of the stream bed will move once bedload transport rates are sufficient to wash out or crush eggs. Within a relatively narrow band on the cross section, corresponding to the width of the portion of the redd in which eggs lie, it may be reasonable to assume that the active bedload transport layer has a uniform thickness that can be referenced more accurately to the mean elevation of the surrounding stream bed surface. Hence, the most useful datum in scour studies appears to be the elevation of the original undisturbed stream bed.

A related source of variability is the horizontal location of the egg pocket within the redd. Egg pockets are generally found in the upstream third of the tailspill, but they may be found in the pit of the redd or farther downstream along the tailspill of long redds with multiple pockets (Hardy 1963; Hawke 1978; Young et al. 1989; Grost et al. 1991). The depth of the egg pocket, relative to either the original gravel surface elevation or to the overlying gravel, can thus vary with position within the redd. Grost et al. (1991) observed that brown trout egg depth increased from the redd pit downstream to the tailspill end. Hawke (1978) noted the same for chinook salmon and postulated that egg burial depth decreased over time as the female expended her energy. Hardy (1963) noted conversely that the first egg pocket was not buried as deep as subsequent pockets. Crisp and Carling (1989) noted that in two redds examined intensively, all egg pockets within a redd were at approximately the same burial depth relative to the overlying gravel surface. Hawke (1978) noted that the number of chinook salmon eggs tended to decrease as successive pockets were created, i.e., were generally greatest in the slightly deeper, downstream pockets of the redd. The data of Hardy (1963) and Hawke (1978) suggest that this form of variability can be on the order of as much as 11 cm for trout and 22 cm for salmon redds.

Such "primary" pockets would certainly be of greatest interest in scour studies should it be determined that loss of shallower, secondary pockets would not lead to excessive loss of eggs and embryos (what is considered excessive still needs to be defined and should factor in the loss of embryos of the adaptive components of a population). The uppermost developing embryos may emerge prematurely or be impacted by fine sediment deposition (Everest et al. 1987; Crisp and Carling 1989). Loss of the uppermost eggs to scour could thus have a negligible influence on production in many instances. However, given the declining status of a large number of salmonid stocks (Nehlsen et al. 1991), it is reasonable to assume that the shallowest pockets may still need to be protected (if possible) to maximize embryonic survival to emergence. It is thus important to know the range of egg pocket depths within the redd and where they are located if the depth datum used is with respect to the overlying gravel. For data reported according to that datum, it is difficult to recalculate the egg pocket elevation with respect to the level of the original stream bed surface.

The reported mean depth of the egg pocket may not always represent actual digging ability because excavation barriers during spawning can limit it. Clay layers can restrict burial

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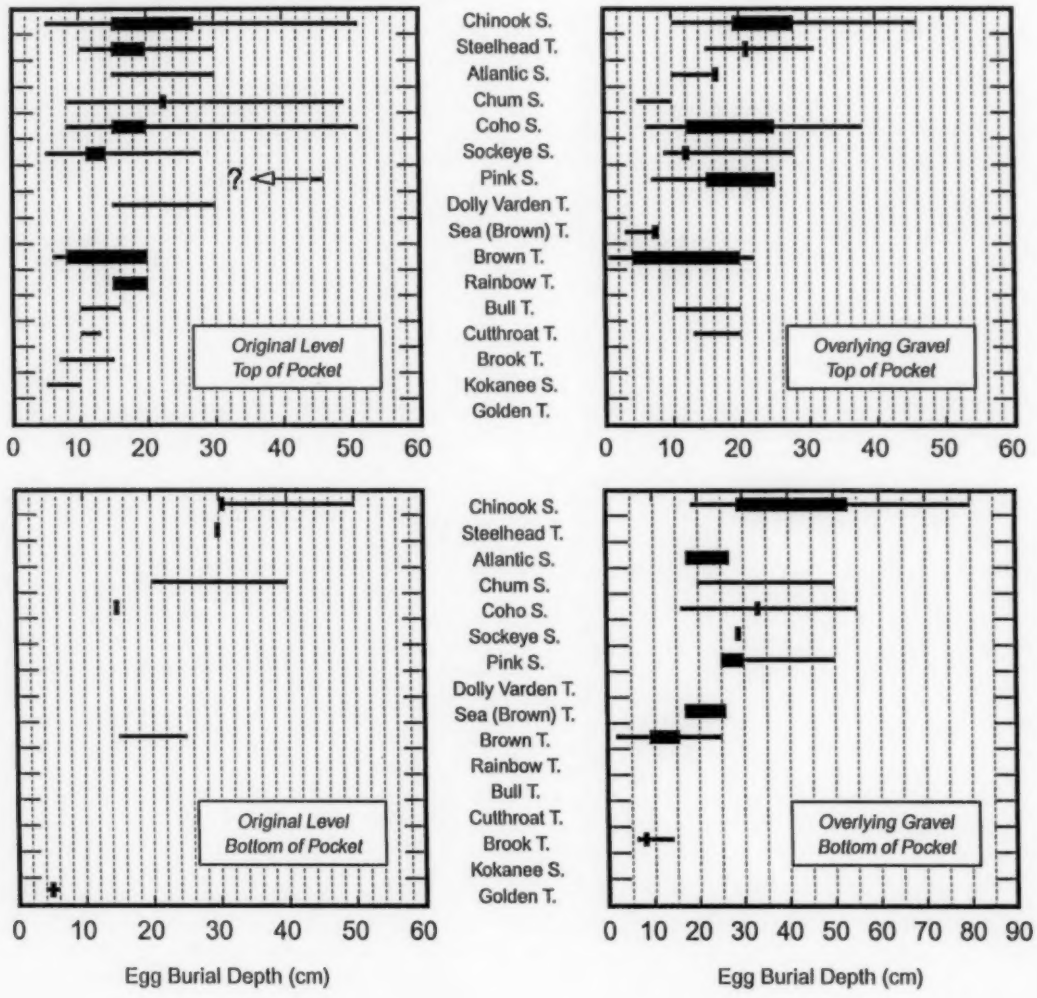
Fig. 2. Summary of egg burial depth data. Species were ordered according to general female size differences and differences in expected egg burial depths assumed a priori. The thin bar depicts the range of data, including depths of discrete eggs; the thick bar the range of different study means, or single observations where no range was available.



depths in completed redds (Barlaup et al. 1994) or induce the female to move elsewhere (Mathisen 1955). Large, flat rocks beneath the stream bed surface can also limit burial depth (Crisp and Carling 1989). The spawning instinct may be so strong that in streams with relatively limited spawning gravel quantities, the female constructs a shallower redd than one in which she might normally spawn (Stuart 1953). Some of the shallower egg depths reported in the literature for a given fish size may have resulted from such limitations, thereby contributing to the overall variance in the data. This is a difficult factor to assess and characterize quantitatively within an equation that could be used to predict egg burial depth. Available data suggest that eggs buried shallower than about 5 cm are likely the result of excavation barriers, excepting the eggs of females smaller than about 150–160 mm in length (cf. Knapp and Vredenburg 1996).

The field method used to determine egg depth can also lead to differences in reported egg depths. Data based on observation of the spawning act can be influenced by parallax, refraction, and observer calibration errors. Crisp and Carling (1989) felt that the freeze-core method gave more accurate and complete estimates of egg depth than manual excavation of in-channel redds. Manual excavation probably does yield less accurate estimates of depth to the uppermost eggs because stream flow may wash away the first few eggs before they are detected. The flow field around the excavation may cause the edges of the excavated pit to cave in, making it more difficult to both see the eggs and estimate the depth relative to the original gravel surface elevation. Nonetheless, excavation-based depth estimates of pockets containing the majority of eggs are likely to be reasonably accurate because there are more eggs to be detected. Use of a McNeil sampler (McNeil and Ahnell 1964) or other device that shields the excavation from the flow field probably gives results similar to the freeze-core

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method. A drawback to the use of freeze corers or McNeil samplers for deriving scour criteria is that the measured datum is typically the overlying gravel and thus varies depending on sampling location on a redd (Fig. 1). However, both are still reasonable approaches if the top of the core sample is indexed against the original, level stream bed elevation. Excavation is practical for sampling stranded redds. Without specific experimental data, the variability introduced by measurement technique is estimated here to be on the order of less than 3–5 cm (possibly more if the tailspill is extremely pronounced), with greatest error inherent in visual observations of excavated redd depths.

Some of the data in Table 1 may have been subjected to varying episodes of scour and (or) aggradation between the times of redd construction and sampling. Scrivener and Brownlee (1989) posited that scour may leave eggs at shallower incubation depths during most of the incubation period than at the time of spawning. Peterson and Quinn (1996) noted scouring of some redds where measured mean depths of egg pocket ceilings decreased from 22 to 19 cm between the fall spawning period and the following spring. Rukhlov (1969) observed a gradual decrease in mean deposition depth of pink salmon eggs over several months after a storm but noted that the event was not a normal flood. Aggradation was also noted, where egg depths were as much as 65 cm (cf. Fig. 2). However, much of the data in Table 1 were collected during or shortly after spawning activity, well before significant stream bed elevation changes could have occurred. Grost et al. (1991) found no significant difference in brown trout egg depths between fall and winter sampling. Furthermore, researchers would likely have known of severe scouring events such as large floods, ice breakups, and significant flood transport of large woody debris and qualified the data accordingly. This source of variability thus was probably not a significant problem for most of the data in Table 1 and Fig. 2.

Mass spawning and repeated, heavy spawning use over many years at the same location may lead to formation of persistent bedforms with maximum dune heights as much as 0.75–1.5 m (Tutty 1986; Everest et al. 1987; Salo 1991). A hole nearly 1 m deep was noted in association with mass spawning of cutthroat trout (Kiefling 1978). Egg burial depths may be much deeper or shallower than in areas with low concentrations of redds, making identification of a consistent egg burial depth difficult. The magnitude relative to the original stream bed elevation could thus be on the order of as much as a meter, but the data in Table 1 suggest that such an occurrence would be relatively infrequent. The hydraulics over such bedforms can be quite complicated, and it is difficult to identify a consistent scour depth within them as well. However, mass spawning activity could reduce bed mobility because of surface coarsening and creation of bedform roughness (Montgomery et al. 1996). Hence it is possible that scour down to egg depths is less of a concern for spawning areas with bedforms than in areas without, but this needs to be confirmed.

Species and microhabitat influences on egg burial depth

The tendency for certain species to bury their eggs deeper than others has been noted frequently since the first published observations of salmonid spawning activity. Greeley (1932) wrote that brown and rainbow trout redd pits were, on average, larger than brook trout pits. Inter- and intra-species variation in egg burial depth may have important survival implications. Variation in spatial and temporal distributions of scour may influence which species can best reproduce and survive in a specific stream (e.g., Kondolf et al. 1991). Tripp and Poulin (1986) collected egg depth data in recently spawned coho and chum salmon redds that suggested the former species buried its eggs deeper than the latter; estimated scour-related loss rates were consequently greater for chum salmon. Reduction in average fish size over time through selective fisheries may lead to reduction or possibly eradication of a stock in streams where the prevailing scour depths favor larger individuals that are able to bury their eggs deeper (van den Berghe and Gross 1984; Montgomery et al. 1996).

Differences between species appear linked to a combination of physical and behavioral factors. Possible sources of variation in egg burial depth include the size of female (e.g., van den Berghe and Gross 1984; Heggberget et al. 1988; Crisp and Carling 1989); her excavation behavior (e.g., Burner 1951; Scott and Crossman 1973; Groot and Margolis 1991; Meehan and Bjornn 1991); and her selection of particular substrate size distributions, velocities, and depths (e.g., Bovee 1978; Kondolf and Wolman 1993). It has also been suggested that differential egg burial depths may reflect differences in egg size and energy reserves between stocks of the same species (Scrivener and Brownlee 1989). Furthermore, egg burial depth appears to be controlled by the character and availability of spawning habitat within a given stream. Streams with substrates that are smaller and less armored, overlapping, or cemented may facilitate deeper redds than streams with contrasting substrate characteristics (e.g., Burner 1951). Egg burial depth may be inversely related to the amount of fine sediments present (Everest et al. 1987), a behavioral response that might improve survival to emergence in some instances.

Most empirical evidence points to a set of specific factors that are best correlated with egg burial depth: fish size (Fig. 2), and local substrate and hydraulic characteristics. Of these, fish size appears to be the most important determinant of maximum egg burial depth. Larger females have greater strength and mechanical advantage over smaller ones (van den Berghe and Gross 1984). Observations and analyses supporting a relationship between fish size and egg burial depth are numerous. Early researchers implied a length-dependent relation by noting that female salmon and trout excavate redds that are longer and deeper than their bodies (e.g., Greeley 1932; White 1942; Scott and Crossman 1973). Others have noted that a proportionality exists between fish size and redd size (e.g., Burner 1951; Shapovalov and Taft 1954). Crisp and Carling (1989) considered the proportionality to be a reflection more of fish size than of species.

Several quantitative relationships have been developed. Ottaway et al. (1981) found a significant semilogarithmic relationship between female fork length and depth to base of main egg pocket for brown trout and Atlantic salmon. Van den Berghe and Gross (1984) also found a significant linear relationship between female fork length and depth to the egg nest bottom for coho salmon. Crisp and Carling (1989) found a linear relation between female length and egg burial depth for anadromous and resident brown trout and Atlantic salmon in some streams but not others; scatter was sufficiently large that regression slopes did not significantly differ from zero, however. Ranges in predicted egg depths, for the range of all fish sizes measured, were on the order of 13–15 cm in all three studies.

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A smaller number of researchers have found little to no relationship between fish size and egg burial depth. Elliott (1984) studied two streams and found no such relation within a specific stream: all egg burial data fell consistently about a mean depth that appeared to be invariant with fish size. However, the anadromous brown trout in one stream were generally larger than the resident brown trout in the other, and the substrates in the former stream appeared to be generally smaller (Elliott 1973, 1984). Average egg burial depth was substantially deeper for the anadromous trout stock, indicating that the trends observed in Elliott's (1984) data could have been linked to differences between not only stocks (which was reflected in fish size) but also substrate and hydraulic characteristics within the two streams.

Any correlation between egg depth and fish size is thus likely to be influenced by variability in local spawning microhabitat parameters. Larger fish or species can use deeper and faster water and larger gravel than smaller individuals (Arnold 1974; Bovee 1978), although larger gravel used by larger fish may counteract the tendency to dig deeper, and a positive correlation between egg size (which in turn is correlated with fish size) and spawning gravel size (Quinn et al. 1995) may partially offset differences in egg settling depth. Of the potential hydraulic and geomorphic features influencing spawning habitat, substrate characteristics appear most influential, followed by velocity. Many researchers have suggested that gravel size characteristics are very important in determining egg burial depth (e.g., Burner 1951; Vasilenko-Lukina 1962; Tautz and Groot 1975; Grost et al. 1991). Heggberget et al. (1988) found that gravel sizes used by Atlantic salmon and brown trout differed significantly. However, there was considerable overlap in the ranges of gravel sizes used, and it is possible that other studies that did not find a relationship did not have sufficiently large sample sizes to detect a statistically significant difference (e.g., Ottaway et al. 1981; Crisp and Carling 1989). Characterizations of the surface layer (e.g., Heggberget et al. 1988) neglect the effects of armoring and variable layer composition (e.g., coarser surface and finer subsurface) on the final redd depth and thus may add variability to results. Given such problems, I was unable to estimate potential magnitudes of variation in egg depths owing to substrate influences from the data.

Experimental evidence for the influence of velocity on egg depth is less conclusive than for substrate. Researchers have made visual correlations between egg depth and velocity (e.g., Vasilenko-Lukina 1962), but the exact relationship remains to be determined. Egg burial depth in faster water may be shallower than in slower water (Vronskii 1972; Neilson and Banford 1983). However, Burner (1951) noted the opposite trend. Combinations of velocity with energy slope and substrate characteristics likely act in concert to determine the depth of the redd. Tautz and Groot (1975) commented that velocity may have a greater influence on redd depth in the initial stages of redd building rather than later. As the redd takes shape, the dominant influence was thought to be the hydraulic force exerted by the female's tail. However, since the female uses her tail to redirect and accelerate higher momentum fluid from the main flow field down into the redd, it is likely that the velocity field is important throughout redd construction.

More data are needed on fish size, substrate characteristics, and the velocity field at the time of redd construction. A large set of consistent data with the same datum and reference frame for the egg pocket boundary would facilitate multiple regressions or more advanced multivariate techniques. An organized approach is recommended in which data on egg burial depth are collected systematically in several streams that characterize a range of substrate and hydraulic conditions. Each stream should ideally contain a range of naturally reproducing salmonid species such that species and fish size differences can be investigated more fully. Fish size appears to be the most important influence on egg burial depth and should be evaluated first, followed by other variables. The power available to the fish in digging a redd clearly must contribute to redd depth, but more studies are needed with larger numbers of females across all size ranges to develop relationships that are more definitive than existing ones. Although the results of investigations into the importance of microhabitat characteristics are to date inconclusive, the substrate grain size distribution of the surface and subsurface layers and the degree of overlap must be important in view of armoring effects and the critical shear and normal stresses needed to dislodge and transport material. Hydraulic principles suggest that the near-bed velocity field in the water column just upstream of the redd is also likely to be important. Water depth and energy gradient influence the shear stress acting at the stream bed at the time redd excavation is begun. However, flow separation at the upstream edge of the pit once the redd is more developed implies that tractive force due to the main flow field is not necessarily a determinant for the eventual egg burial depth, and water depth at the time of redd construction would therefore not be expected to correlate strongly with redd depth (as has indeed been the case to date). Relationships between egg burial depth and fish species and size therefore need to be developed that include adjustments for substrate (primarily) and velocity (secondarily) characteristics but not necessarily water column depth.

A final source of variability is related to the egg pocket thickness, which is likely scaled to the size of the larger substrate particles present. Mathisen (1955) noted that egg pockets of Alaskan sockeye salmon were approximately 10 cm thick, while F. Everest (personal communication cited in Chapman (1988)) observed that the majority of eggs in chinook salmon redds in Oregon lay in a stratum 2–3 cm thick just above the undisturbed surface of the stream bed. Peterson and Quinn (1997; N.P. Peterson, Simpson Timber Co., Shelton, WA 98584, U.S.A., personal communication) observed that chum salmon egg pockets were usually about 10 cm thick. Crisp and Carling (1989) found that the majority (85–90%) of eggs in 40 Atlantic salmon and brown trout redds were aggregated within ±2–3 cm of the mean burial depth. Barlaup et al. (1994) found that Atlantic salmon and sea (brown) trout egg pockets were 6.4 and 8.0 cm thick on average, respectively. The data suggest that the larger, anadromous salmonids tend to bury their eggs in a pocket that is approximately 8–10 cm thick and smaller trout in a pocket that is approximately 6–8 cm thick, but more work is needed in this area. The thickness of the pocket has implications to the vertical distribution of eggs (Holtby and Healey 1986) and the total mortality to scour. For the same pocket bottom depth, eggs buried in a narrower vertical distribution could suffer lower total egg loss than eggs buried in a thicker layer as long as the maximum scour depth was still above the bottom of the egg pocket.

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Egg depth criteria for scour evaluations

Egg burial depth data evidently must be interpreted with care. Data presented by Montgomery et al. (1996) suggest that measurement errors in pocket depths of a few centimetres could influence survival predictions significantly for a particular depth of substrate disturbance. Individual sources of variation can contribute to differences in egg depths up to 15 cm or more. Cumulative variation, due to measurement error and unexplained sources, could easily be on the order of 5–20 cm in most studies, depending on species and the other factors discussed above.

A different source of variation not included in this analysis that may influence scour mortality predictions stems from downward movement of alevins through the substrate after hatching (Bams 1969; Dill 1969; Fast et al. 1981). However, alevin movement generally may not happen until more than halfway through the intragravel life-history phase (e.g., see individual species reviews in Groot and Margolis 1991). Scour down to the egg burial depth will impact developing embryos until then. Scour depth criteria developed from egg depth data are expected to provide a conservative level of protection for alevins in consideration of their mobility.

Pending additional research, I offer suggested preliminary threshold criteria for scour studies in Table 2. The criteria were developed in consideration of the data compiled in Table 1 and summarized in Fig. 2 and of expected size differences between species. They were based primarily on the range of reported mean values, which was assumed to approximate the depth at which the majority of eggs may be found. The criteria are biased towards smaller females because insufficient data exist for developing consistent size-dependent criteria. The reader is referred to the studies of Ottaway et al. (1981), van den Berghe and Gross (1984), Holtby and Healey (1986), and Crisp and Carling (1989) for preliminary quantitative relationships of egg depth and fish size; I recommend drawing conditional conclusions concerning scour impacts from those relationships. The criteria that are proposed here are in many cases based on limited data and should change as better data become available. In the meantime, the values in Table 2 for the top of the egg pocket are appropriate for estimating the onset of scour impacts, while the criteria for the bottom of the pocket can be considered as conservative estimates of the point at which the population will become decimated because of scour. Such information should prove useful for modeling studies that link hydrology and sediment inputs to scour and incubation survival, although assumptions would still need to be made concerning the distribution of eggs between the two limits (e.g., Holtby and Healey 1986; Scrivener and Brownlee 1989).

For improved threshold criteria, a more consistent approach is needed wherein depths are measured to both the top and the bottom of each egg pocket, relative to the original stream bed surface elevation. Regression analyses may be more useful in scour studies if based on the lower envelope of egg depth data rather than the mean of the data. Such threshold-type relationships could be used to predict the onset of scour-related mortality of incubating embryos. It should be possible to develop functional relationships for the minimum or maximum depths of the egg pocket that would be expected for a given fish size (or, say, 10 or 90 percent regression quantile; cf. Terrell et al. 1996). Because a few loose eggs are often deposited close to the surface, a less variable relationship may be determined for the distance down to the top of distinct egg pockets rather than the first few eggs encountered. An egg pocket could be defined as containing five or more eggs (cf. Crisp and Carling 1989; Tripp and Poulin 1986, Fig. 6) separated in space by no more than a few egg diameters. A drawback to threshold criteria is that they indicate only when impacts may occur rather than how much.

Table 2. Preliminary egg burial depth criteria proposed for use in scour studies.

	Depth (cm) below original stream bed level	
Species	Top of pocket	Bottom of pocket
Atlantic salmon	15	30
Brook trout	5	15
Brown trout	8	25
Bull trout	10	20
Chinook salmon	15	50
Chum salmon	15	35
Coho salmon	15	35
Cutthroat trout	10	20
Dolly Varden trout	15	30
Golden trout	3	6
Kokanee salmon	5	15
Pink salmon	15	35
Rainbow trout	10	25
Sea (brown) trout	10	25
Sockeye salmon	10	25
Steelhead trout	15	35

Note: Criteria are proposed as maximum allowable depths of scour before initial ("top") and total ("bottom") egg loss.

Frequency distribution data for both egg pocket depths and for discrete eggs within the pocket are more useful for estimating scour-related loss rates (e.g., Mathisen 1962; Tripp and Poulin 1986; Montgomery et al. 1996). Data for chum salmon in Kennedy Creek in western Washington State indicated that egg pocket ceiling depths were distributed lognormally (Montgomery et al. 1996), where the median depth was smaller than the mean value by approximately 2 cm (i.e., skewed right; Peterson and Quinn 1996). However, data for discrete coho and chum salmon eggs collected by Tripp and Poulin (1986) indicated a skewed-left lognormal distribution. The two results suggest that interpretations of scour impacts will vary depending on the form in which egg depth data are presented. The frequency data for depths down to the top of egg pockets suggest that scour depths approximating the mean value would result in impacts to more than half of the total number of redds created. Conversely, the frequency data of Tripp and Poulin (1986) suggest that scour depths approximating the mean depth of discrete egg burial would result in the loss of less than half the eggs. Both views are important because they provide information on impacts to genetic variability and total production, respectively.

More comprehensive relationships should be developed that describe the frequency characteristics of egg burial depth. Ideally, egg depth distributions should be developed for different size-classes of females, for each species. Frequency data need to be established for both the top of the egg pocket and for discrete eggs. Such data would facilitate comparisons of

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predicted egg burial depths with spatial and temporal frequency distributions of scour depth for spawning runs composed of different-sized individuals and species. Management decisions regarding land-use practices influencing sediment supply and flood hydrology, fish size and species or stock harvest restrictions, hatchery brood stock characteristics, and escapement goals could all benefit. Decisions that consider egg depth frequency characteristics may ultimately prove to be more effective for preserving specific salmonid stocks than decisions based on threshold conditions, because they would be based on the goals of protecting both genetic variability and population size from scour impacts.

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"Risk" in fisheries management: a review

R.I.C.C. Francis and R. Shotton

Abstract: "Risk" has appeared more frequently in the fisheries management literature in recent years. The reasons for this are partly internal (scientists seeking better ways to advise fishery managers) and partly external (e.g., adoption of the precautionary approach). Though terminology varies, there is consensus that there are two stages in dealing with risk. The first (here called risk assessment) is the formulation of advice for fisheries managers in a way that conveys the possible consequences of uncertainty. This advice is in the form of an evaluation of the expected effects of alternative management options, rather than recommendations. Risk assessment has been undertaken in many fisheries, and there is general agreement as to how it should be done (although technical details differ). The second stage (risk management) is the way fishery managers take uncertainty into account in making decisions. Much fisheries risk management is informal, i.e., nonquantitative, undocumented, and loosely linked (if at all) with a risk assessment. The major reason for this is that the objectives of fisheries management are often conflicting and are rarely stated in a way that provides explicit direction to managers or scientists.

Résumé : Le mot risque est employé plus fréquemment dans la documentation sur la gestion des pêches au cours des dernières années. S'il en est ainsi, c'est en partie pour des raisons internes (les scientifiques cherchent de meilleures façons de conseiller les gestionnaires des pêches) et en partie pour des raisons externes (p. ex., l'adoption d'une approche prudente). Bien que la terminologie varie, il y a consensus sur le fait qu'il existe deux niveaux lorsqu'on parle de risque. Le premier niveau (appelé ici évaluation du risque) est la formulation d'un conseil à l'intention des gestionnaires des pêches d'une manière qui met en lumière les conséquences possibles de l'incertitude. Ce conseil prend la forme d'une évaluation des effets prévus de différentes options de gestion, plutôt que de recommandations. L'évaluation du risque a été entreprise dans le cas de nombreuses pêcheries et, de façon générale, on s'entend sur la façon dont elle doit être effectuée (bien que les détails techniques varient). Le deuxième niveau (gestion du risque) est la façon dont les gestionnaires des pêches tiennent compte de l'incertitude lorsqu'ils prennent des décisions. Une grande partie de la gestion du risque dans le domaine des pêches est informelle, c'est-à-dire, non quantitative, non documentée et liée vaguement à une évaluation du risque (si jamais elle l'est). La principale raison qui justifie cette situation, c'est que les objectifs en matière de gestion des pêches sont souvent contradictoires et qu'ils sont rarement formulés d'une manière qui donne des instructions explicites aux gestionnaires ou aux scientifiques.
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Introduction

Since the beginning of the 1990s there has been increasing use of the word "risk" in documents concerned with the management of fisheries. Although many uses have been nontechnical (i.e., the word has been used in one of its normal English meanings) increasing numbers of authors have used the word in a technical sense. That is, they have assigned a specific, sometimes quantitative, meaning to their use of the word (sometimes in compound forms such as "risk analysis," "risk assessment," or "risk management").

There has also been an increased institutional interest in risk in relationship to fisheries management. Several conferences have focussed, in whole or in part, on this topic (SEFSC 1991 (cited in Caddy and Mahon 1995); NAFO 1991; Smith et al. 1993; International Counicil on the Exploration of the Sea (ICES) theme session on risk, Dublin, 1993), and some analysis of risk has become an important tool in many major fisheries forums, e.g., in ICES (Serchuck and Grainger 1992; Kirkegaard et al. 1995), the International Whaling Commission (IWC) (Kirkwood 1993), and the National Marine Fisheries Service (NMFS) (Rosenberg and Restrepo 1994).

Three recent papers have commented on aspects of this growing literature. Shotton (1993) discussed the use of the concepts of risk, uncertainty, and utility; Rosenberg and Restrepo (1994) summarized applications in U.S. marine fisheries; and Caddy and Mahon (1995) discussed risk and uncertainty in relation to reference points in fisheries management. The present paper is intended to extend these commentaries in a broader review. Its aim is to discover how the concept of risk is being used in the fisheries management literature, to show where consensus occurs, and, where it does not, to describe the debate. This paper is intended for a broad audience and so does not delve into mathematical or statistical details.

Much of the literature reviewed here might be considered to fall under the heading of fisheries science, rather than fisheries management, and so it might be asked why we have used the latter term in our title. The answer is that a major reason for the increasing use of "risk" in this literature appears to have been a desire on the part of fisheries scientists to improve their advice to those who manage fisheries.

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R.I.C.C. Francis.[1] National Institute of Water and Atmospheric Research, P.O. Box 14901, Wellington, New Zealand.
R. Shotton. Marine Resources Service, Food and Agriculture Organization of the United Nations, Via delle Terme di Caracalla, 00100 Rome, Italy.

[1] Author to whom all correspondence should be addressed.
e-mail: c.francis@niwa.cri.nz

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Why risk?

Before examining how the concept of risk has been applied in fisheries management it is worth asking why it has been applied. There appear to be three main reasons: the perceived failure of fisheries management in recent decades, changing public attitudes to risk, and increased computing power.

The failure of fisheries management

It is widely felt that modern fisheries management has seen many more failures than successes (Stephenson and Lane 1995, and references therein). Thompson (1993) listed 13 stocks that have "collapsed" recently (in the sense that they have experienced a severe decline in biomass and have subsequently failed to recover, despite a reduction in the fishing mortality rate). Horwood (1993), speaking of European fisheries, stated that "many of our most important stocks are at historically low levels." (In these cases, failure is evident; we shall see below that it is not always easy to determine whether management has failed because the objectives of management are often not stated.)

This perceived failure has focussed attention on how institutions involved in fisheries management operate. In particular, it has caused fisheries scientists to examine both the form of their advice to fishery managers, and how, or whether, that advice has been used. One consequence has been the realization of the importance of incorporating into advice some expression of the uncertainty associated with it:

> "Understanding the risk or uncertainty associated with choices could help fishery managers select management strategies, decide which types of risks and uncertainties inhibit the effectiveness of management techniques, and finally, recognize which types of uncertainty must inevitably remain as a part of the fishing business." (Peterson and Smith 1982);
>
> "With an appropriate understanding of the risk of severe decline of the fishery relative to the biological productivity of fish stocks, managers can integrate socioeconomic and political information to make final decisions." (Linder et al. 1987);
>
> "The managers' task may be made easier if uncertainty in a fishery assessment were expressed in terms of risk to the fishery..." (Francis 1992);
>
> "Scientific advice to fishery managers needs to be expressed in probabilistic terms to convey uncertainty about the consequences of alternative harvesting policies." (McAllister et al. 1994);
>
> "Clearly, when management decisions are to be based on quantitative estimates from fishery assessment models, it is desirable that the uncertainty be quantified, and used to calculate the probability of achieving the desired target and/or risk of incurring undesirable events." (Caddy and Mahon 1995).

Rosenberg and Restrepo (1994) also noted that, for some fisheries, stock assessments are highly controversial and political, with a number of nations and (or) interest groups being involved. In such situations, some form of risk analysis or assessment may be useful to allay possible concerns about the scientific advice "by confronting uncertainty directly."

Changing public attitudes to risk

The general public are becoming increasingly aware of the extent to which industrial activities can have major impacts on the environment and human health. As a consequence, they are demanding greater regulatory controls over these activities. Major events such as the Exxon Valdez oil spill, the Chernobyl and Three Mile Island nuclear power generation accidents, and the proliferation of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes of North America have caused the public to demand more caution on the part of regulators. A recent editorial in *Nature* (7 March 1996) highlights the increasing role of "science-based risk analysis" in environmental legislation in the United States.

Walters and Pearce (1996) believe that these trends will lead to greater public influence in fisheries management decisions, and that "this influence will pressure fisheries agencies to adopt low-risk policies." Two manifestations of the public desire for more caution in fisheries management are the *602 Guidelines* in the United States and the promotion of the precautionary approach. Both have had the effect of focussing the attention of fisheries scientists and managers on questions of risk.

The 602 guidelines

These guidelines were published by the United States Department of Commerce in 1989 and require fishery management agencies to "specify, to the maximum extent possible, an objective and measurable definition of overfishing for each stock or stock complex" (quoted in Mace and Sissenwine 1993). Further, they require that recovery plans be formulated for all stocks that, according to these definitions, are found to be overfished (Rosenberg and Restrepo 1994).

The precautionary approach

The precautionary approach has been embodied in national laws and regulations for many years, particuarly in matters relating to human health and, more recently, the environment (Garcia 1994*b*). For example, in the regulation of pharmaceuticals, this approach requires that new products may not be sold until they have been shown to pose no unacceptable risk. More recently, following its adoption in Principle 15 of the Rio Declaration of the UN Conference on Environment and Development in 1992, interest has been renewed in applying the precautionary approach to fisheries management (Garcia 1994*a*, 1994*b*).

A precautionary approach to fisheries management was first advocated as early as 1955 (Kesteven and Holt 1955) and interest in the concept has developed internationally, spurred on by initiatives on the part of FAO (the Food and Agriculture Organization of the United Nations). The precautionary approach played a part in the UN Conference on Straddling Fish Stocks and Highly Migratory Fish Stocks (Garcia 1994*a*); it has been adopted as one of the general principles to be followed in a code of conduct for responsible fisheries (FAO 1995*a*), and guidelines on its application have been drawn up (FAO 1995*b*). These guidelines note that "Management according to the precautionary approach exercises prudent foresight to avoid unacceptable or undesirable situations, taking into account that changes in fisheries systems are slowly reversible, difficult to control, not well understood, and subject to change in the environment and human values."

Given the precedent of the UN Convention on the Law of the Sea, it seems likely that the precautionary approach will soon become part of the national laws and international treaties

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governing the management of fisheries in many parts of the world.

Increased computing power

The techniques described below for quantifying risk are conceptually simple but require very large numbers of calculations. Until recently, computers sufficiently powerful to perform these calculations were not available to many fisheries scientists. As Efron (1979) has noted (in reference to the development of computer-intensive statistics) the increase of computing power has allowed analysts to "think the unthinkable." However, as Caddy and Mahon (1995) point out, it is still true that "the cost and availability of information and expertise required may preclude the use of these techniques for many small or low value stocks and for most stocks in developing countries."

Uncertainty

There is a general consensus in the literature on risk in fisheries management that risk, however it is defined, arises from uncertainty. The most useful definition of uncertainty, in this context, seems to be "The incompleteness of knowledge about the state or processes (past, present, and future) of nature" (after FAO 1995*b*). Thus, it is agreed that it is a lack of knowledge that causes risk.

Some authors have used more specific or technical definitions of uncertainty. Peterson and Smith (1982) distinguished between risk, which they viewed as a "measurable probability..." and uncertainty "which is subjective". Shotton (1993) also referred to this distinction (which he said is one used by economists) and noted (citing Lindley 1985) that, in decision theory, "uncertainty" refers only to unknown events in the future. However, these distinctions do not appear to have been found useful by many authors in the fisheries literature.

Error and uncertainty

Before discussing categories of uncertainty, we will comment on the relationship between "error" and "uncertainty." These are allied concepts but are not, as some authors have treated them, equivalent. Although error implies uncertainty (e.g., error in measurement leads to uncertainty about the measured quantity) the reverse is not true. Another important source of uncertainty is natural variation. This is often confused with error, as the following example illustrates.

Consider a scatterplot of body mass versus body length for some species of fish. It is useful to summarize the relationship between these two quantities by some curve that passes through the middle (in some sense) of the scatter of points. In using regression to derive an equation for this curve we commonly refer to the deviation between any individual point and the line as "error." This may be convenient, but it is misleading. It implies that the deviation may be partitioned into error in length and error in mass, whereas the natural between-individual variation that causes the great majority of the scatter in this plot (we assume measurement error is minor) cannot be subdivided in this way. That is, it is not sensible to ask whether a point that lies above the curve does so because the associated fish is heavy for its length, or short for its mass. If we use the scatterplot to infer the mass of an individual (not represented in the plot) from its length, there will be uncertainty in our inference. This uncertainty derives from natural variation, not error.

Six types of uncertainty

A number of authors have categorized the types of uncertainty (sometimes called error) that are important as sources of risk in a fisheries setting (A.D.M. Smith 1993; Megrey et al. 1994; Rosenberg and Restrepo 1994; Caddy and Mahon 1995; Hilborn and Peterman 1996; Fogarty et al. 1996). Six types have emerged: those associated with process, observation, model, estimation, implementation, and institutions. We will discuss these in turn.

Process uncertainty has been defined as "the underlying stochasticity in the population dynamics such as the variability in recruitment" (Caddy and Mahon 1995, following Rosenberg and Restrepo 1994) or "random variation in demographic rates and processes" (Fogarty et al. 1996). This type of uncertainty arises from natural variability, not error. For that reason it seems desirable to avoid the term "process error." Although this term is readily understood amongst specialists, it may be confusing, and misleading, in communications with fishery managers and stakeholders. The most common example of process uncertainty in the fisheries risk literature is interannual variability in recruitment. Beddington and Cooke (1983) tabulated estimates of the extent of this variability for a large number of fish stocks.

Observation uncertainty arises in the process of data collection, through measurement and sampling error (the latter deriving from the fact that we observe only a sample from a population, not its entirety). For data on commercial and recreational fisheries, inadequate data collection systems and deliberate misreporting may also be significant sources of observational uncertainty.

Model uncertainty arises from the "lack of complete information on the population and community dynamics of the system" (Fogarty et al. 1996). The term "model" refers to the conceptual model that fisheries scientists and managers use as an aid in making inferences and decisions about fish populations and fisheries. Sometimes the models are qualitative and the uncertainties are unquantified (e.g., uncertainties about the location of stock boundaries and whether fish prices will rise or fall). More commonly, the models are mathematical, i.e., a set of equations describing (a simplified version of) how populations and fisheries change over time. For this type of model the uncertainty includes lack of information about the correct structure (e.g., is the stock–recruit relationship asymptotic or domed?) and parameter values (is natural mortality 0.2 or 0.3?). Note that the distinction between structure and parameter values is arbitrary. The structural question of whether to use an asymptotic (e.g., Beverton and Holt) or domed (e.g., Ricker) stock–recruit relationship may be made into a parametric question simply by using a more complicated stock–recruit function which can take either shape.

An important, and easily overlooked, type of model uncertainty is that to do with what is commonly called the "error structure" (a structure that often includes both error from observations and natural variability!). Inferences drawn from modelling exercises may be profoundly affected by the choice of error structure (see Schnute (1991) and Polacheck et al. (1993) for examples).

Estimation uncertainty relates to the process of parameter

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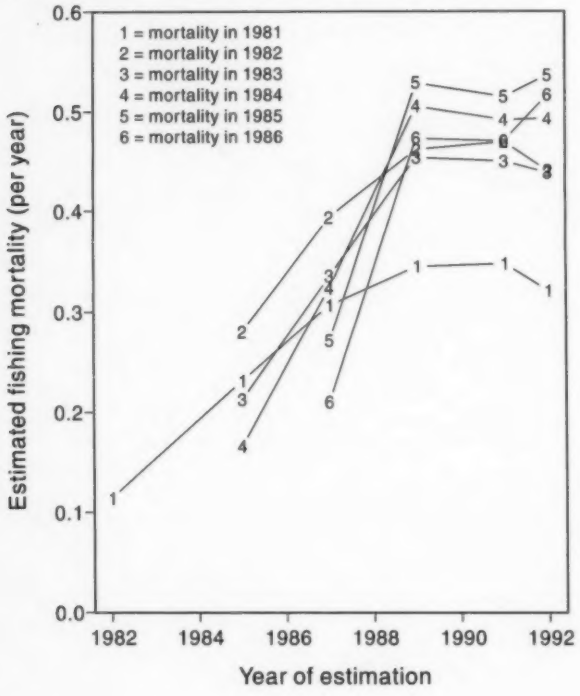
Fig. 1. Illustration of the retrospective problem in the analysis of catch-at-age data. Sequences of successive estimates of instantaneous fishing mortality for 7-to 9-year-old cod in Labrador and northeastern Newfoundland (NAFO region 2J3KL). Note that each sequence shows a trend in the same direction (upward). More information about these data is given by Myers et al. (1997).



estimation. It is a secondary type of uncertainty in that it derives from some or all of the three above types. Estimation requires data and a formula, or algorithm, which implies a model. The former are subject to observation uncertainty and possibly process uncertainty (e.g., if the data are numbers of recruits), the latter to model uncertainty. A striking, and important, example of the possible effects of estimation uncertainty is the so-called "retrospective problem" in the analysis of catch-at-age data (Fig. 1; Sinclair et al. 1991; Parma 1993).

Rosenberg and Brault (1993) drew attention to implementation uncertainty, by which they meant uncertainty about "the extent to which management policies will be successfully implemented." For example, a target harvest rate (the management policy) may not be achieved. They noted that the risk associated with alternative management policies depends in part on how effectively they are likely to be implemented. Rice and Richards (1996) suggested that management actions were likely to be ineffective when the objectives of management and industry differ substantially. Angel et al. (1994) concluded that the primary cause of the failure to conserve groundfish stocks on the Scotian Shelf off eastern Canada between 1977 and 1993 was a failure in implementation: "In sum, the tactical approach chosen to control fishing mortality generated illegal [fishing] behaviour which was not curbed by the available enforcement regime."

O'Boyle (1993) defined institutional uncertainty as arising from "... problems associated with the interaction of the individuals and groups (scientist, economist, fisherman, etc.) that compose the management process" and suggested that this could exceed "quantifiable" sources of uncertainty in stock assessments. One type of institutional uncertainty is associated with the lack of well-defined social, economic, and political objectives in fisheries management. Participants in a recent workshop identified this as a major source of uncertainty (S.J. Smith 1993). Serchuck and Grainger (1992) and Francis (1994) describe institutional settings in which, because clear management objectives have not been stated, scientists have had to try to infer them. Megrey et al. (1994) stated that "the communication of risk to fisheries management by scientists is hindered by a lack of well-defined objectives for fisheries management." Stephenson and Lane (1995) claim that part of the reason for many fisheries failures in recent decades is that "objectives have been broad, ill-defined, and in many cases not operationally feasible."

Reducible and irreducible uncertainty

Fogarty et al. (1996) made an important distinction. They noted that uncertainties derived from observations and models (and thus from estimation) are, in principle, "reducible" by, for example, more intensive sampling and increased research effort. Institutional and implementation uncertainties are also, with appropriate effort, reducible. However, process uncertainty is inherent and thus "irreducible." For example, with increased effort one may measure recruitment more precisely, and learn more about why it fluctuates, but whatever is done (short of extinguishing the population) will not stop it fluctuating.

The terminology of risk

There has been some debate in the literature about appropriate technical definitions of terms like "risk" and "risk analysis." In commenting on this debate we will follow the suggestion of Shotton (1993) that an important criterion is "how well do such terms help managers to make the 'best' decision?"

Risk

There are two schools of thought on how "risk" should be defined and used in the fisheries management literature. The great majority of authors have, whether explicitly or implicitly, taken it to mean something like "the probability of something undesirable happening" (e.g., Peterson and Smith 1982; Brown and Patil 1986; Bergh and Butterworth 1987; Linder et al. 1987; Swartzman et al. 1987; Hall et al. 1988; Francis 1991; Punt and Butterworth 1991; Fogarty et al. 1992; Restrepo et al. 1992; Hilborn et al. 1993; FAO 1995*b*; Fogarty et al. 1996). Others have preferred "decision-theoretic" definitions; i.e., those that treat risk as an expected loss and thus incorporate both the probability and the severity of the undesirable event(s) (Shotton 1993; Horwood 1993; Rosenberg and Restrepo 1994). This is the interpretation of risk used in some formulations of decision theory (Ferguson 1967; Berger 1985).

We cannot turn to common English usage to resolve this debate because both meanings occur. For example, most people, in interpreting the phrase "the risks associated with smoking are high," would consider both the severity and probability

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of consequences (a severe consequence that has a very low probability, e.g., death in an airplane crash, does not constitute a high risk). However, in the phrase "the risk of death is high," "risk" would be taken as synonomous with "probability."

A major reason that most authors have not used decision-theoretic definitions may be the great difficulty involved in quantifying the severity of certain "undesirable" events (Horwood 1993; Mohn 1993). For example, although it is widely believed that a low level of spawning biomass is undesirable (because it may lead to stock collapse), we are aware of no attempt to estimate the severity (in the form of potential losses) of this event (see The Risk of Collapse, below).

Because of this difficulty it seems sensible to follow FAO (1995*b*) in treating risk as "the probability of something undesirable happening," and reserving the terms "expected loss" or "average forecasted loss" for quantities encompassed by decision-theoretic definitions. Note that this is simply a matter of terminology. It is not to say that we should ignore the issue of severity; this should be quantified wherever possible. Of course, to make the above definition operational we must be more specific (see Choice of Performance Measures, below).

Two stages in dealing with risk

Although their terminology varies, many authors appear to accept the view that the process of dealing with risk in fisheries management has two distinct stages. The first deals with the formulation of advice for fisheries managers; the second deals with the ways in which managers use that advice to make decisions. We will follow Pearse and Walters (1992) and Lane and Stephenson (1997) in calling these "risk assessment" and "risk management," respectively.

Lane and Stephenson (1997) quote from the decision analysis literature to support their contention that the term "risk analysis" should be used to describe the combination of these two stages. This broad use of "risk analysis" has also been advocated in the area of animal health (Ahl et al. 1993; they add a third stage: risk communication). However, many authors in the fisheries literature use this term to refer to what is here called risk assessment.

Risk assessment

The aims of risk assessment may be encapsulated in two quotes. Peterson and Smith (1982) commented that it is useful "to evaluate possible management techniques as they may increase or decrease risk and uncertainty for the fishing industry and for achieving stated objectives of management." Fogarty et al. (1996) said that "Formal risk assessment provides a mechanism for explicitly accounting for uncertainty in framing fishery management advice."

The following quotations illustrate the range of ways in which the first stage in dealing with risk has been defined:

> Risk analysis is "the evaluation of the probability of an end event or events happening which result from a combination of events" (Brown and Patil 1986);
>
> Risk assessment "deals with methods of assessing the probability of possible outcomes of decisions" (Pearse and Walters 1992);
>
> "Risk assessment or analysis..., as seen from the fisheries scientists' point of view, most often focuses on ways of quantifying probabilities of outcomes, and on ways of fixing acceptable levels for these probabilities." (Basson 1993);
>
> "Risk analysis may be thought of as being comprised of two components. The first is the propogation of uncertainties for a given course of action and the second is a metric of consequences." (Mohn 1993);
>
> Risk assessment "... has been used to mean estimating the probability that a given management decision or strategy will exceed some defined management threshold." (A.D.M. Smith 1993);
>
> Risk evaluation is ways "to pass on the interpretation of [the effects of uncertainty] in a constructive manner to the fisheries managers and fishing industry" (S.J. Smith 1993);
>
> "Risk analysis is the evaluation of benefit streams under uncertainty produced in a risk assessment using a specified loss or utility function." (Rosenberg and Restrepo 1994);
>
> "Risk assessment is the process that evaluates possible outcomes or consequences and estimates their likelihood of occurrence as a function of a decision taken and the probabilistic realization of uncontrollable state dynamics of the system.... [it] assigns probabilities to the multidimensional simulation outcomes for each decision alternative" (Lane and Stephenson 1997).

Although the authors variously use the terms "risk analysis," "risk evaluation," and "risk assessment," they are all, with one exception, defining more or less the same activity. That is, "using information on the status and dynamics of the fishery to present fishery managers with probabilistic descriptions of the likely effects of alternative future management options." The one exception is that part of the definition by Basson (1993) that refers to "fixing acceptable levels"; most authors would place that activity in the second stage, as part of risk management. Note that the distinction made by A.D.M. Smith (1993) between risk assessment and management strategy evaluation is avoided by this definition.

Risk management

In the fisheries context, risk management has been defined as the following:

> "[dealing] with ways of responding to uncertainty about outcomes" (Pearse and Walters 1992);
>
> "the process of making of decisions concerning risks and the subsequent implementation of those decisions" (Basson 1993);
>
> "Application of decision-making criteria embodied in management utility functions that measure the expected value of each decision alternative in terms of the multiple criteria and their tradeoffs, and thereby evaluates and ranks alternative decisions for presentation to decision makers" (Lane and Stephenson 1997).

We feel that none of these definitions is sufficient and thus satisfactory. The first two are general, providing no direction for those concerned with the application of a methodology. The last is an improvement in at least dealing with expected values of uncertain outcomes but fails to say that the response of decision makers will depend on their attitude to risk (aversion to, or propensity for).

Of course, any decision process that may result in an undesirable outcome and that takes uncertainty into account, in some way or other, may be termed risk management. However, there

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Table 1. Key components in fisheries management applications of risk assessment.

Inputs	(1) Data on the fishery and the fish population (including estimates derived from such data) (2) A model describing the dynamics of the fishery (3) Quantitative descriptions of uncertainty about the data and (or) the model (4) Several alternative future management options
Outputs	One or more performance measures describing the future performance of the fishery under each of the alternative management options.
Method	Monte Carlo projection

Note: See text for details.

is a broad spectrum of possible approaches to risk management, ranging from the informal to the formal. On this spectrum, the great majority of risk management in fisheries would lie close to the informal end. This sort of management is characteristically nonquantitative, undocumented (in terms of decision criteria and process), and only loosely linked (if at all) with a risk assessment. It may be imagined as taking the form of negotiations in a smoke-filled room.

Formal risk management entails a description of the decision criteria (i.e., those to be used in choosing amongst alternative management options) that is sufficiently complete and specific to define the quantities that should be calculated in the risk assessment and to make the decision, given the results of the risk assessment, clear cut. The key point here is that the decision criteria should be stated, very specifically, before the risk assessment takes place.

Note that, even with the most formal of risk management, it will often be unclear in advance what range of alternative management options to include in the risk assessment. There will always need to be scope for the risk assessment to be repeated with different management options until an acceptable one is found. In addition, as Morgan and Henrion (1990) note, rarely is a problem solved once and for all. However, once an acceptable management option is found, it should be, according to the decision criteria, preferable to all other options considered. (We take the view that the occurrence of two or more equally acceptable options is unlikely in practice, no matter how diverting it may be in theory).

Risk assessment in practice

Although there is no agreement in the fisheries literature about how we should define risk assessment, and what we should call it, there appears to be broad agreement on the type of analysis that should be done. In this section we first present a schema that describes this area of agreement and then discuss some issues that arise from an examination of recent practice.

Common components

Almost all fisheries management applications of risk assessment have certain components in common (Table 1), which we will discuss in turn.

The inputs

Examples of data inputs include catch and effort (either of which may be broken down by area, time, vessel type, etc.); survey estimates of population abundance; estimates of rates of growth and natural mortality; etc.

The descriptions of uncertainty will include at least one of the six types described above and will contain uncertainty about both the current and future status of the fishery. Two types of descriptions are used. The first is probabilistic, taking the form of statistical distributions (e.g., giving the range of values a parameter might take and the probability associated with each value). The second type consists of a set of alternative hypotheses to which no probabilities are attached. For example, there may be two different explanations for recruitment fluctuations (Parma and Deriso 1990) or several alternative data sets (Punt and Butterworth 1991).

The management options are typically to do with the annual decisions which affect the level of catch in the fishery, through controls on either catch or effort. They may be only for the next year (i.e., the first year in the future), or may extend many years into the future (in which case the word "strategy" may be preferable to "option"). We will refer to the period covered by these management options as the "management period." Two distinct classes of management options are described below.

The model is usually in the form of a computer program that describes, in a simplified way, the dynamics of the fishery (i.e., how the population changes over time with different levels of fishing).

The method

Monte Carlo projection is a way of dealing with uncertainty in evaluating management options. If there were no uncertainty the model could produce, for each alternative management option, a single exact description of the future of the fishery (Fig. 2A). Because there is uncertainty, the method of Monte Carlo projection produces a large number (usually at least 100, often 1000 or more) of alternative possible futures for the fishery for each management option (Fig. 2B). Each of these possible futures (often referred to as "realizations") is a description of how the fishery might develop as a result of that option.

Note that what constitutes a realization depends on how complex the model is. For a simple model, a single realization would consist of just the population biomass and the catch for each year of the management period. With a more complex model, the biomass and catch might be specified for each age-class, and the catch may be further broken down by area and (or) vessel type. In some assessments, each realization will also extend into the past and thus describe a possible past for the fishery. However, because we are evaluating future management options, our focus will be on the future part of each realization.

The word "projection" refers to the fact that the method projects the fishery into the future. The label "Monte Carlo" signals that there is a random element in the way the realizations are constructed. For example, suppose that, in the situation illustrated in Fig. 2B, the only uncertainties are (*i*) the

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present biomass and (*ii*) the recruitment to the fishery (which may or may not depend on spawning biomass) in each of the 5 years of the management period. Each of these uncertainties would be described by a probability distribution (i.e., a function describing the probability of each of the possible values of biomass or recruitment). To generate one realization the computer program must first pick six random numbers, one from each of the six probability distributions. This set of six numbers describes a possible reality. For this possible reality the model will produce, for each management option, one realization.

Note that the uncertainty is expressed by the difference between alternative realizations; there is no uncertainty in a particular realization (but see below the distinction between quantifiable and unquantifiable uncertainty). The greater the uncertainty, the greater the range of possible futures described by the realizations. Note that in Fig. 2B most of the biomass trajectories increase. Thus, we could say that, under option O_1, the biomass will probably increase. If the projections are done in such a way that each of the realizations is considered equally likely, and if the biomass increases in 80 of 100 realizations, we would say that there is a probability of 0.8 that the biomass will increase. In some risk assessments there will be a different probability associated with each realization.

The outputs

The performance measures are numerical descriptions of the likely future effects of the alternative management options, taking into account uncertainty about the current status of the fishery and its future behaviour. Typical examples are the probability that the biomass will fall below some threshold level during the management period, the expected catch over that period, and the expected net present value of the catch. Performance measures are often thought of as expressions of "risk" in one or another of the senses defined above.

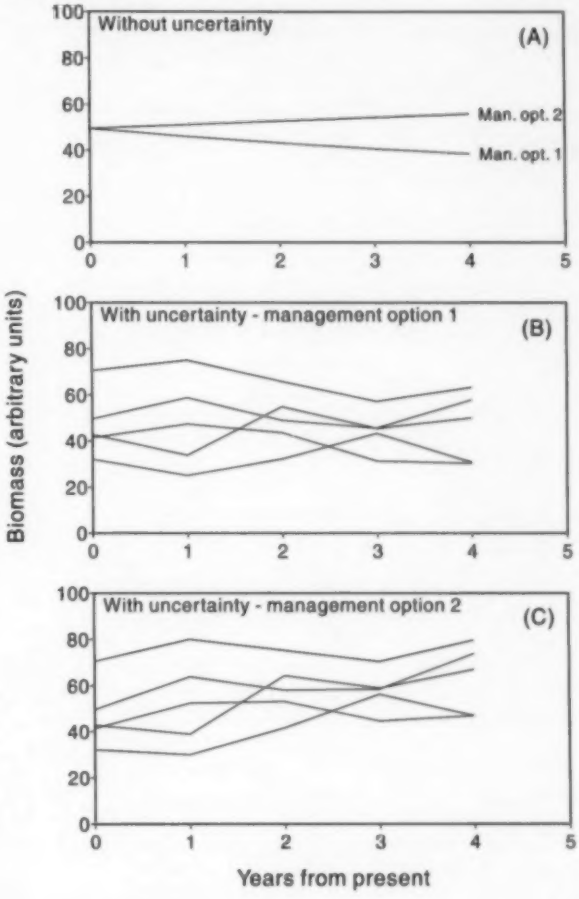
Note that all we know about the possible future effect of a particular management option is contained in the set of realizations (possible futures) calculated for that option in the projections. Thus, the performance measures may be seen as summaries of this information. A key point about them is that they are typically statistical in nature, e.g., probabilities, expected values, standard deviations, etc. They summarize the range of possible futures for each alternative management option.

Two types of risk assessment

Risk assessment has been applied to fisheries management in two distinct situations, which we might label "stock assessment" and "harvest strategy evaluation." What distinguishes these applications is whether they take into account the current status of a particular fish stock. The former type of application does take this into account and is aimed at helping managers to decide what actions to take in the immediate future in managing that stock. In this situation it is of great importance to know whether the stock is currently depleted or not, and whether recruitment in the next few years is likely to be high or low. Examples of this type of risk assessment are given by Brown and Patil (1986), Mohn (1991), Francis (1992), Mesnil (1993), and McAllister et al. (1994).

In stock assessment applications the management period (as defined above) is generally short (1–5 years). It is important to realize that the management period is not the same as what we might call the "decision period," the time period covered by the decision that managers have to make (the decision for which the stock assessment is intended to provide supporting information). Most fisheries management is done on an annual cycle so the decision period is generally only 1 year. Francis (1994) used the phrase "Think five years, act one year" to make an analogy with the environmental dictum "Think global, act local" in explaining the philosophy behind making the management period longer than the decision period. Most people, institutions, or goverments can act only on a local level, but the environmental dictum reminds them of the importance of considering the global implications of their actions. Similarly, there are a number of constraints (chiefly a lack of information) that require fishery management actions to be annual, but it is often prudent to consider the longer term implications of making particular annual decisions. For this reason the management period may be longer than the decision period.

Fig. 2. Illustration of the method of Monte Carlo projection for two alternative management options over a 4-year management period. Plots of biomass against time: (A) without uncertainty, one curve for each option, and (B and C) with uncertainty, five realizations (alternative possible futures) under management options 1 and 2, respectively.



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Harvest-strategy evaluations ignore the current status of a fish stock and are not concerned with immediate management decisions. They are aimed at answering the question "What would happen in the long term if we used a specific rule (harvest strategy) to make our annual management decisions?" Here the management period is longer, typically 10–50 years. The harvest rules may be simple (e.g., take the same catch, or the same proportion of the biomass, each year) or more complicated (e.g., strategies that change when the biomass falls below a specified threshold). Examples of harvest-strategy evaluations are given by Ruppert et al. (1985), Swartzman et al. (1987), Hall et al. (1988), Francis (1993), Sigler and Fujioka (1993), Megrey et al. (1994), and Kirkwood (1997).

Within harvest-strategy evaluations, two types of questions are considered: "What are the pros and cons of different types of strategy?" and "What are the effects of different levels of fishing intensity for a given strategy?" For example, how do the consequences of catching the same amount of fish each year (constant catch strategy) compare with those from taking the same proportion of the population each year (constant fishing mortality)? For the latter strategy, what are the effects of different levels of fishing mortality?

There is a technical difficulty with this sort of risk assessment in that the fishery must be given some sort of initial state for the stochastic projections, but it is desired that the results should not depend on that intial state. A common solution to this problem is to make the period covered by future projections somewhat longer than that used in calculating the performance measures. For example, Megrey et al. (1994) projected 70 years into the future but used only the last 50 years in calculating their performance measures.

Some authors have used the term "management procedure" to encompass both a harvest strategy and descriptions of the data that should be collected each year and the methods used to analyse these data to calculate the harvest level. Risk assessments that evaluate management procedures (e.g., Butterworth and Bergh 1993; Punt 1995; Kirkwood 1997) must simulate not only the fish population, but also the collection and analysis of data. Kirkwood and Smith (1996) discuss how such analyses may be used to assess how precautionary a proposed management procedure is likely to be.

The problem of presentation

Although the general approach to risk assessment in fisheries (Table 1) is commonly accepted, Rosenberg and Restrepo (1994) noted that "there is, as yet, no standard approach in the presentation of advice [to fishery managers] with respect to uncertainty and risk." Further, the development of effective methods of presentation "presents substantial challenges to both fishery technicians and managers" (Caddy and Mahon 1995). Two issues to be decided are which performance measures to use, and how complex should the presentation be for each performance measure.

Choice of performance measures

There is little agreement as to the what are the "best" performance measures. To some extent this is inevitable in that the choice of measure should depend on the objectives of the managers of the fishery in question and their attitude to risk. Also, for stock assessments, it may depend on the current status of the fishery; performance measures appropriate for a developing fishery may not make sense for one that is overexploited. However, in considering the very wide range of performance measures that have been used it is hard to escape from the conclusion that it would be useful to narrow this range.

A bewildering variety of performance measures have been used. We counted approximately 39 different performance measures in a sample of about 20 published risk assessments. (These numbers are approximate because it was sometimes debatable as to how many separate risk assessments are contained in one paper, and some performance measures were not sufficiently well described to indicate whether they were distinct from others.) To give some idea of how it is possible to generate so many different measures it is useful to present a formal description of the choices involved (Table 2).

In step 4 of Table 2, some authors, rather than presenting a single number, chose to present the whole distribution (Fig. 3). (Note that Fig. 3B is equivalent to calculating $P(B < B_{\text{targ}})$, where P is the probability and B is the biomass, for all possible values of the target biomass, B_{targ}.)

The description in Table 2 is sufficiently general to cover most performance measures in the fisheries literature. One example not covered is that of net present value (NPV). Calculating this type of performance measure involves determining the monetary value of the catch and then applying an appropriate discount rate (discount rates are considered further below).

One of the most common performance measures is of the form $P(B < B_{\text{thr}})$, where B_{thr} is the threshold biomass (see Table 2). However, opinions differ as to the appropriate level for B_{thr}. The most popular value is probably $0.2B_0$ (which seems to originate from Beddington and Cooke (1983), where B_0 is the virgin biomass), but a range of other values have been used: $0.1B_0$ (Berg and Butterworth 1987), $0.25B_0$ (Hall et al. 1988), $0.5B_{\text{MESY}}$ (Getz et al. 1987; B_{MESY} is the biomass associated with the maximum estimated sustainable yield), the minimum historic value (Sigler and Fujioka 1993), and the biomass for which the mean recruitment is estimated to be half the maximum mean recruitment (Restrepo and Rosenberg 1994) (also, see Myers et al. 1994). Nor is there any consensus as to whether this probability should be be calculated as a proportion of years or of realizations (some authors do not even clearly specify which is used). (In the formalization of Table 2 this is equivalent to deciding whether, at step 2, the five values of B for each realization should be summarized by their minimum value.)

There are three criteria that should be considered in choosing a performance measure. First, it should be readily intelligible to managers and other stakeholders. In our experience, standard deviations and coefficients of variation are not easily understood by nonspecialists (despite their importance in statistical inference) and are best avoided. For describing variability in catch, the average absolute change in catch from year to year is probably more intelligible. (We note though that many papers using standard deviations or coefficients of variation are aimed at scientists, who may be more able to interpret these quantities than fishery managers or other stakeholders.) Second, a performance measure should show contrast between alternative management options. If it takes almost the same value for all management options it is of little use in decision making. Third, it must be related to management objectives. This should, of course, be the first criterion. We have given it

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Table 2. Description of four choices that must be made in constructing a single performance measure. To clarify the presentation we assume that the risk assessment has involved calculating 100 "possible futures" (realizations), each projecting 5 years into the future.

1. Which "attribute"?	Most examples use catch, C; biomass, B; fishing mortality, F; or recruitment, R. For each choice this results in a set of 500 numbers (5 years times 100 realizations). Some performance measures have used the change in catch from year to year, ΔC.
2. Summarize within realizations?	For example, rather than using all five catches for each realization, it might be better just to use the mean, minimum, or total or to use only the catch in the final year. If this summary is made, the set of 500 numbers reduces to 100.
3. What units?	For example, rather than dealing with a biomass in tonnes it might be preferable to divide by the virgin biomass, B_0, or some target biomass, B_{targ}, and thus deal with biomass as a fraction of B_0 or B_{targ}.
4. Which summary statistic?	Generally the performance measure is a single number summarizing the set of 500 (or 100) numbers. For example, the mean, standard deviation, coefficient of variation, median, or some other percentile (e.g., 5th or 95th). Alternatively, it may be expressed as a probability, e.g., the probability that the biomass will fall below some threshold level, written $P(B < B_{thr})$.

Note: More complicated performance measures require additional choices (see text).

last because it is probably the most difficult; management objectives are usually not stated explicitly, and when they are, the statements are often too vague to be useful in constructing a performance measure.

Complexity of presentation

The complexity of the presentation will depend to some extent on the nature of the uncertainties. The simplest presentation is of the type shown in Table 3A. For each management option, a single number is presented for each performance measure. However, such a simple presentation will only be possible if all the uncertainty can be expressed probabilistically.

When some of the uncertainty is expressed in the form of alternative hypotheses a presentation like Table 3B may be appropriate. Sometimes one of these alternatives will be preferred (or considered most likely) and may be referred to as the "base case" assessment. The other assessments are then considered as sensitivity analyses. Suppose, for example, that the alternative hypothesis differs from the base case by changing one model assumption. Then the sensitivity of the assessment to this assumption is measured by how much the calculated values of the performance measures change between the two analyses. In some assessments there will be no base case, i.e., none of the alternative hypotheses will be considered "most likely."

Note that, in presentations like that in Table 3B, uncertainty has been dealt with in two ways. The "quantifiable uncertainty" (i.e., all that to which probabilities can be assigned) has been incorporated into the stochastic projections. It is expressed as the difference between realizations and is summarized by the performance measures. The "unquantifiable uncertainty" is that incorporated in the alternative hypotheses. This is expressed, for each management option, by the amount of variation between the calculated values of each performance measure.

Some authors have advocated presentations like that in Table 3C (e.g., Hilborn et al. 1993, Table 2; McAllister et al. 1994, Table 6; Hilborn and Peterman 1996, Table 2). Here, part of the quantifiable uncertainty has been disaggregated and expressed as a set of alternative "states of nature" with probabilities

Fig. 3. Example of two ways of presenting a performance measure (from the risk assessment in Fig. 2) as a distribution, rather than a single number. Each graph describes the uncertainty the biomass 4 years from the present for each of two management strategies: (A) as a probability density and (B) as a cumulative distribution function.

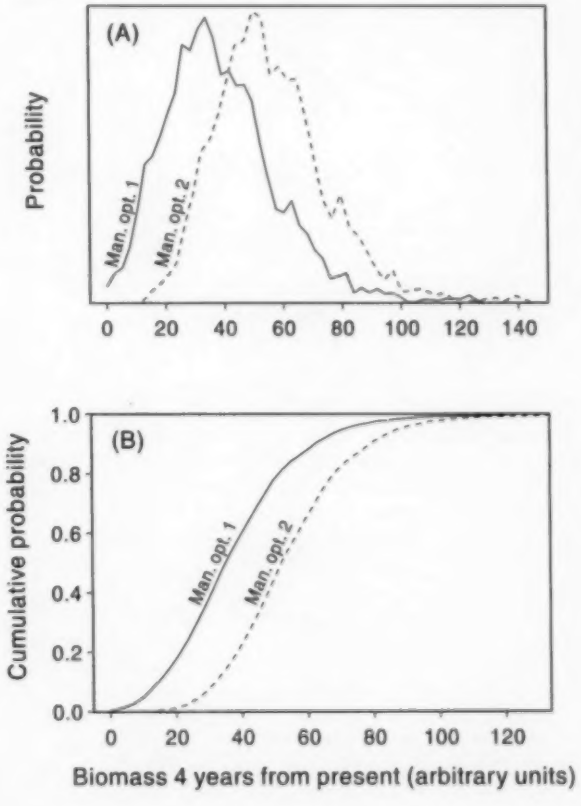

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Table 3. Example of three levels of complexity in the presentation of results from a risk assessment.

(A) Simplest case

	Performance measure	
Management option	$P(B < B_{thr})$	Mean C
Option 1	0.1	1000
Option 2	0.2	1200
Option 3	0.3	1500

(B) With two alternative hypotheses

Management option and hypothesis	Performance measure	
	$P(B < B_{thr})$	Mean C
Option 1		
Base case	0.10	1000
Alternative hypothesis 1	0.05	900
Alternative hypothesis 2	0.02	700
Option 2		
Base case	0.20	1200
Alternative hypothesis 1	0.15	1150
Alternative hypothesis 2	0.12	950
Option 3		
Base case	0.30	1400
Alternative hypothesis 1	0.25	1200
Alternative hypothesis 2	0.23	1000

(C) With alternative states of nature

	$P(B < B_{thr})$				Mean C			
Recruitment:	Poor	Medium	Good	Expected	Poor	Medium	Good	Expected
Probability:	(0.3)	(0.4)	(0.3)	value	(0.3)	(0.4)	(0.3)	value
Management option								
Option 1	0.35	0.15	0.01	0.17	600	900	1500	1000
Option 2	0.50	0.25	0.05	0.27	700	1200	1700	1200
Option 3	0.70	0.33	0.1	0.39	900	1600	2000	1500

assigned to each alternative. Note that the expected value columns in Table 3C are equivalent to the columns in Table 3A. Of course, if there are also alternative hypotheses, Table 3C will become more complicated again.

This type of presentation involves some difficult choices for the analyst. First, which portion of the quantifiable uncertainty should be disaggregated? In the two examples quoted above it was uncertainty about B_0. It could just as easily have been uncertainty about the natural mortality, M. Second, if the range of possible values for the variable concerned is inherently continuous (as it is for B_0 and M), how is it best divided into discrete alternative sets of values? For example, what should be the boundaries between "low," "medium," and "high" B_0, and would it be better to have only two categories or perhaps four?

In choosing between methods of presentation like those in Table 3 the main question that must be answered is "Which will be most likely to lead to the best decision?" Table 3A would seem ideal: a single compact table that, if the performance measures are well chosen, clearly summarizes the likely performance of the fishery under alternative management options. Unfortunately, there are often unquantifiable uncertainties so the next level of complexity (Table 3B) is often unavoidable. A challenge for analysts is to restrict the number of alternative hypotheses so as to adequately express the uncertainty without providing an overwhelming number of alternatives. It is unclear whether the complexity added by the disaggregation in Table 3C will lead to better decisions, especially if there are unquantifiable uncertainties to be dealt with so that a combination of Tables 3B and 3C is necessary. Clearly there needs to be close collaboration between those who carry out risk assessments and those who use them to ensure that the form of presentation used aids the decision-making process to the greatest extent possible.

True versus perceived states

A number of authors have stressed the importance in risk-assessment models of distinguishing between the "true" and perceived states of the fishery (Punt and Butterworth 1991; Hollowed and Megrey 1993; Rosenberg and Restrepo 1993; Kirkwood 1997). This may involve having a single model and two sets of parameters or two different models. In either case, one model (or parameter set) is taken as describing the "true" state of the fishery, and the other, how it is perceived by scientists and managers.

We have written "true," rather than true, here because no model can precisely describe the actual fishery. This is taken for granted. However, it is important to realize that, even if the fishery were described exactly by some model, there would still be an important difference between the actual state of the

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fishery and its perceived state. This arises because of observation and model uncertainty.

Some examples will illustrate the value of this distinction. Hollowed and Megrey (1993) described an assessment in which the proportion of the time that the biomass, B, is greater than some threshold, B_{thr}, (i.e., $P(B > B_{thr})$) is an important performance measure. They showed how it could happen that the perceived value of $P(B > B_{thr})$ may be consistently lower than the "true" value. (Note that B and B_{thr} are parameters that have both "true" and perceived values, the latter being estimates of the former). Rosenberg and Restrepo (1993) examined a similar situation in more detail. They calculated what they called (*i*) "power" and (*ii*) "type I error." These are the probabilities that it will be perceived that B is below B_{thr}, (*i*) given that it actually is, and (*ii*) given that it is not. Punt and Butterworth (1991) discussed a fishery for which there were a number of alternative descriptions (combinations of model and data set). They then showed, for each pair of descriptions, what would be the effect of managing the fishery if one description was assumed to be true while the other was actually true. As a result, they were able to eliminate some descriptions on the basis that they performed so poorly if one or more of the other descriptions were "true."

The value of simple models

There is a natural tendency, as our knowledge of fish and fisheries increases, to incorporate this knowledge in our models and thus make them more and more complex. This would appear to be a positive trend. It seems obvious that the more closely our models imitate reality, the more useful they will be to us. However, recent work has shown that this is not necessarily true (Punt 1993; Steinshamn 1993; Kirkwood 1997). The point is that, in a fisheries management setting, our objective is to manage the fishery well, not to model it well. The above references provide examples where simpler models support fishery management better than more realistic ones.

The risk of collapse

Probably the most extreme "undesirable event" that fisheries managers wish to avoid is the collapse of the fishery. By "collapse" we mean a severe decline in biomass and subsequent failure to recover, despite a reduction in the fishing mortality rate (following Thompson 1993, who gave a table of 13 stocks to which this has happened). Such an event implies a long-term severe drop in production and thus revenue.

It would seem obvious that we should guard against such a catastrophe by making the probability of collapse one of our performance measures in all risk assessments. This way, any management option that produced an unacceptably high probability of collapse could be avoided. Unfortunately this is not possible because we don't understand enough about how fisheries collapse to model this event. This is not to say there are no theories on this subject. The problem is that, for a particular fish stock, we often don't know how to choose between the theories and usually have scant knowledge about model parameter values.

A common response to this lack of knowledge is to use $P(B < B_{thr})$ as a performance measure, rather than P(collapse). The rationale behind this is the belief that the probability of collapse in the near future is inversely related to biomass, with the probability becoming significant as the biomass falls below B_{thr}. Note that this does not mean that the stock will definitely collapse if $B < B_{thr}$, only that the probability of collapse is thought to be significant. However, we are unable to say how high this probability is or how fast it increases with decreasing biomass. In other words, although our Monte Carlo projections allow us to estimate the probability of the undesirable event ($B < B_{thr}$), we cannot estimate its severity. This is in contrast to the undesirable event of fishery collapse, for which we can estimate severity but not probability.

Risk management in theory

Although some form of risk assessment has become routine in the management of many fisheries, there appear to be very few examples where formal risk management (as defined above) has been applied in fisheries. However, there is a growing literature about how it ought to be, or could be, applied. In this section we will discuss first some issues that arise from this literature and then two attempts at formal risk management.

Objective or loss functions

The simplest way to formalize risk management is to devise a single performance measure for the fishery and then choose the management option that maximizes (or minimizes) this measure. The function that calculates this performance measure from a set of realizations is called an "objective function" (if it is to be maximized) or a "loss function" (if it is to be minimized). (In what follows we will refer only to objective functions because a loss function can always be turned into an objective function by taking its negative.)

A slightly more complicated approach would be to maximize one performance measure subject to some constraint on a second measure. Thus, for example, Francis (1993) used mean catch (C) as an objective function and searched for the management option that maximized this subject to the condition that $P(B < 0.2B_0) < 0.1$.

Objective functions have been calculated for various fishery management problems (e.g., Ruppert et al. 1985; Quinn et al. 1990; Hollowed and Megrey 1993; Megrey et al. 1994). However, these have typically been by way of example, rather than operational functions that have been used to make fishery management decisions. Before considering why this is so, we will briefly discuss two technical issues that are of importance in constructing objective functions: utility and future discounting.

Utility

In a fisheries context, some obvious units in which to measure gains (or losses) are tonnes for catch or dollars (or other monetary unit) for income or profit. However, the problem is that these are not necessarily the best units for use in objective functions because they may not measure well the strength of people's preferences. For example, the possibility of obtaining an additional 1 t of catch is generally more attractive to the fisher who already has access to 20 t, than it is to one with 100 t. A second example deals with peoples' attitudes to uncertainty. Suppose we are given the choice between receiving $1000 or tossing a coin and accepting $2000 if it comes down heads and nothing if it comes down tails. In terms of expected outcome, measured in dollars, it would appear that the two options are equally attractive. The fact that many people would

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prefer the certainty of the first option shows us that, at least in this case, dollars are not a good measure of preference.

The concept of utility was devised to deal with this problem. A utility function is a means of expressing outcomes in units that measure preference. When there is uncertainty, it is also a means of expressing the attitude of people (e.g., decision makers) to risk. In the above example, people who prefer the coin toss are termed risk preferrers; those who prefer the $1000 are risk averse. It is clearly important to ensure that the attitudes to risk of decision makers (and (or) stakeholders) are incorporated in whatever objective function is used in a fisheries risk assessment.

It is beyond the scope of this paper to delve further into this complex topic. Readers are referred to Shotton (1993) and Lane and Stephenson (1997) for further discussion in a fisheries context (and references in both these papers for wider applications). Examples of applications of utility functions to fisheries problems are given by Powers and Lackey (1976), Keeney (1977), Mendelssohn (1982), Die et al. (1988), Quinn et al. (1990), Kope (1992), and Horwood (1993).

Future discounting

It is commonly observed that the perceived value of some future benefit depends on how long it is before that benefit is to be obtained (with the value generally declining with increasing time). This is what is called "future discounting," which Shotton (1994) defined as "the concept of time preference in relation to future benefits." The conventional way to deal with this is to express all values in present terms, using a discount rate. For example, the present value of $1000 that is available in 5 years' time is $\$1000/(1 + x/100)^5$, where x is the discount rate (expressed as a percentage). The bigger x is, the more the future is discounted (i.e., the less is the present value of a given future benefit).

The relevance of future discounting to fisheries management in general was clearly illustrated by Clark (1976) and Silvert (1977) who showed that, under certain conditions, if the discount rate of decision makers with regard to future benefits from a fish stock is high enough, there is no economic reason to conserve the stock. In the construction of objective functions for fishery management, discount rates will clearly be relevant for any management options that cover more than 1 year. However, although they have been used and discussed in many theoretical fisheries papers (e.g., Charles 1983; Plourde and Bodell 1984; Hannesson 1986; Horwood 1987; Welch and Noakes 1991; Megrey et al. 1994; Ianelli and Heifetz 1995) their explicit use in fisheries management decisions appears to be rare.

Where the precautionary approach to fisheries management is adopted it would seem that discount rates would have application only in short-term management decisions. For decisions with long-term consequences (e.g., those referred to in the above references to Clark (1976) and Silvert (1977)) the issue of intergenerational equity, which is so central to the precautionary approach (Garcia 1996), would seem to preclude the use of discount rates. For example, even a relatively low discount rate of 2.5% implies that the importance of each successive generation declines by a factor of almost 2 ($=1.025^{25}$, assuming a human generation time of 25 years).

It is important to be sure what is being discounted. Suppose, for example, you are offered a choice between receiving either $750 today and $750 tomorrow, or just $1000 today. If you choose the latter you are likely to be judged as having a high rate of future discounting. However, it could be that the reason for your choice is that you consider money offered today to be certain but that you have doubts as to whether that promised for tomorrow will actually materialize. In other words what you are discounting is not the future value of money, it is the likelihood of receiving it. Now, consider a common situation in which fishers voice a preference for a management option that gives them a high catch in the next year followed by a period of lower catches, over an option that provides medium catches over the whole period. Does this preference stem from their discounting the future value of catches (or money), or are they actually discounting the stock assessment (i.e., the prediction that a high catch next year will need to be followed by a period of low catches)? One might deal with the former type of discounting by incorporating a discount rate in the objective function; this would not be appropriate with the latter type.

Multiple objectives

The objectives of fisheries management are usually multiple and often conflicting. Shotton (1994) noted that "One example FAO often encounters is the desire of countries to earn foreign exchange through exports of fish and at the same time to promote national food consumption of domestic supplies of cheaper fish... Other common mutually-exclusive objectives that are simultaneously sought by Departments of Fisheries are those of economic efficiency and regional development, or equity of income distribution."

Hollowed and Megrey (1993) provide a good example of how conflicting objectives can be combined into a single objective function. Their objective function may be written as

$$\frac{\text{mean}(C)}{\text{MSY}} - \text{P}(B < B_{thr})$$

i.e., it is the mean catch, divided by the maximum sustainable yield (MSY), minus the probability that the biomass falls below a threshold biomass. This function implies two potentially conflicting objectives: to maximize mean catch and to minimize the probability of low biomass. The difficult task in combining these objectives is to decide what is an appropriate trade-off between these objectives. This requires obtaining from fishery managers an answer to the question "How much would you be prepared to allow $P(B < B_{thr})$ to increase in return for an increase in mean catch of 1% MSY?" Hollowed and Megrey have implicitly, and arbitrarily, assumed that the answer to this question is 1%. There is no reason to believe that fishery managers would agree that this was the appropriate trade-off. This objective function could also fail to meet the requirements of fishery managers if there was some level of $P(B < B_{thr})$ that they were unwilling to exceed, regardless of any short-term gain in catch.

There is a formal theory of decision making with multiple objectives (e.g., Keeney and Raiffa 1976), and there have been some theoretical fisheries applications (e.g., Walker et al. 1983; Sylvia and Enriquez 1994). However, it seems that this theory "still remains untried in operational divisions of departments responsible for fisheries management" (Shotton 1993). Pearse and Walters (1992), while admitting the appeal of these techniques as a way of clarifying objectives and attitudes to risk, suggest that "... [they] are not so well developed and

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standardized that they can be routinely applied and readily understood. Nor are the results easily interpreted and evaluated in the usual arenas of political debate."

Eliciting objectives

It has been said above that a major barrier to effective fisheries management is the lack of explicit objectives. This affects both the advice that is offered to support management decisions and the decision-making process. Scientists providing management advice are often forced to infer management objectives so as to formulate their advice appropriately and then may have great difficulty in determining how their advice has been used. Without explicit objectives it is not possible to make rational decisions or evaluate past decisions.

However, the difficulty of eliciting objectives that are sufficiently explicit to be usable in assessing and managing risk should not be underestimated. We use the word "elicit" here to emphasize that this task necessarily involves (lengthy) dialogue between those charged with formulating policy and objectives and those with the skills to express these objectives in an operational form. This is a difficult and time-consuming process. The following quotes identify two related problems.

> "For different fisheries or jurisdictions the decision maker might be a council of ministers, treaty commissioners, the minister of fisheries, a management council or advisory committee, or a fishery manager. The accessibility of the decision maker for questioning about specific management objectives will vary greatly across this range." (A.D.M. Smith 1993);

> "... it is often difficult to identify the decision maker whose attitude towards risk should be the basis for assessing the utility function...Ministers of fisheries come and go, threatening inconsistency over time; external advisers and bureaucrats raise questions about representativeness and accountability; resource users cannot always be relied upon to represent the broader public interest in long-term conservation." (Pearse and Walters 1992)

In jurisdictions where fisheries are managed by councils whose members represent a range of interest groups, individual councillors may not feel it in their interest to be frank about their objectives (because, for example, this could reduce their flexibility in negotiations).

Two attempts at formal risk management

A good example of an attempt at formal risk management is provided by the management procedure for South African anchovy *Engraulis capensis* (Bergh and Butterworth 1987; Butterworth and Bergh 1993; Butterworth et al. 1993). This procedure specifies precisely what data will be gathered each year and the algorithm that will be used to calculate catch limits from these data. In other words, it automates the annual harvest rate decision. The risk management consisted of finding the best values for the parameters of this algorithm. This decision was made after consideration, by managers and fishers, of the results of an extensive series of simulations based on various alternative parameter values. (Thus, this was a harvest-strategy evaluation, as defined above.) The main consideration appeared to be to find an acceptable trade-off between having a high mean catch and not having too much year-to-year variability in catch (anchovy, like most other small pelagic species, fluctuates considerably in abundance from year to year).

This management procedure has been applied in only 4 of the 9 years since it was introduced, despite having been modified twice. In the other 5 years, the catch limit produced by the procedure was deemed unacceptable. Thus, it would appear that the risk management was not very successful. It appears to us to be a good example of how difficult it is to accurately elicit objectives from decision makers. For example, in the original risk assessment it was made quite clear to decision makers that, in a small proportion of years, the procedure would set a zero catch limit because of low stock size. This appeared to be accepted. Yet the first time this occurred the procedure was rejected. It would seem that what appears acceptable on paper, or a computer screen, may not be when it actually occurs.

Kirkwood (1997) provided a very interesting account of another attempt at formal risk management: the development of the Revised Management Procedure (RMP) for the International Whaling Commission. This involved a long (7 years) and exhaustive series of simulation exercises in which a strong emphasis was placed on constructing "robust" procedures. That is, the procedures were expected to perform reasonably well even when the assumptions on which they were based were false. For example, simulations were carried out in which abundance estimates (assumed by the management procedure to be unbiased) were actually biased, either upwards or downwards or with a trend in the bias. The main performance measures used in evaluating candidate management procedures were concerned with interannual variability in catch, minimum population size over the simulation period, total catch, and catch at the end of the management period. No attempt was made to combine these performance measures into a single objective (or utility) function, it being judged "unlikely that such a utility function could be derived."

It remains to be seen how successful this risk management will be, because, although it led to agreement on the form of the RMP, the procedure has yet to be applied because the IWC moratorium on commercial whaling is still in force.

The division of responsibilities

Throughout this review we have repeatedly referred to two groups of people: (fisheries) scientists, and decision makers (or managers). A number of authors have commented on the roles for these two groups, either as these have evolved over time, or as it is felt they ought to be to facilitate effective fisheries management.

There is a broad consensus that it is the job of scientists to carry out risk assessments (i.e., to evaluate alternative management options) and of decision makers to do risk management (i.e., to make decisions based on the assessed risks) (Pearse and Walters 1992; Hilborn et al. 1993; Morrissey 1993; A.D.M. Smith 1993; Fogarty et al. 1996). This may seem too obvious to be worth stating, but a glance backwards in time shows that roles have not always been so distinct. In this context Serchuck and Grainger (1992) and Kirkegaard et al. (1995) present a very useful description of how the activities of ICES have evolved since 1976. ICES is an exclusively scientific body, founded in 1902, that provides scientific information and advice on environmental and fisheries management to a

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range of management bodies. It was not until 1991 that it specifically recognized that it was not its role to define management objectives, but rather it should "present options as to how management objectives can be reached and... clearly describe the implications and consequences of these options and their associated risks" (Serchuck and Grainger 1992).

A.D.M. Smith (1993) drew a clear distinction, stating that the role of scientists is to (*i*) elicit and clarify objectives, (*ii*) turn objectives into specific attributes and criteria, (*iii*) identify a range of strategy choices, (*iv*) evaluate outcomes, and (*v*) communicate the results to the decision maker; and that of the decision makers is to (*i*) specify the objectives of the management, (*ii*) evaluate the results and weight the objectives, and (*iii*) make the decision.

Hilborn et al. (1993) made a similar distinction but suggested two specific limits on the role of scientists (the "stock assessment group"). The first is that they should make no recommendations. This is consistent with the trend for scientific bodies to evaluate alternatives rather than make recommendations. However, ICES still reserves the right to recommend management actions for stocks that are "at or below the 'minimum biologically acceptable level' (MBAL) or expected to become so in the near future at current levels of fishing mortality rate" (Kirkegaard et al. 1995). Second, scientists should not attempt to make "best" estimates of biological parameters such as MSY or current stock size.

Rosenberg and Restrepo (1994) disagreed with this, stating that it often requires scientists' special knowledge about species, stocks and models to determine what the best estimate is. However, this may be a misunderstanding. What Hilborn et al. (1993) appeared to be saying was that scientists should not hide uncertainty by presenting only a best estimate. For example, the uncertainty inherent in conflicting data should be clearly presented in the form of alternative estimates. Sometimes it would be appropriate to label one of these estimates as best (i.e., most likely).

Caddy and Mahon (1995) expanded on the above role of the decision makers saying that they "must develop means of objectively evaluating the potential costs of undesirable events and define acceptable levels of risk and of short-term yield which can be foregone to reduce these risks."

The need for extensive dialogue between the two groups is emphasized. In the first place, this is necessary to clarify objectives. This will be an ongoing process because objectives will change as new situations emerge and the membership of decision-making bodies, and governments, change. The trend to increase the participation of the fishing industry and other stakeholders (recreational fishers, environmental groups, indigenous peoples) in the decision-making process makes this an increasingly challenging task. Second, decision makers will often want to consider management options not included in an initial risk assessment. Hilborn et al. (1993) and Walters (1994) favour providing them with the means, in the form of computer programs, to evaluate alternative strategies themselves.

Lane and Stephenson (1995) take a somewhat different view from those stated above. They contrast fisheries science, which they say has "established a large literature and a strong scientific methodology" with fisheries management, which, "in contrast to fisheries science, has not developed a standardized methodology.... [it] has not formally considered the full suite of implications that its policies engender, and as a result it has failed to live up to its broad mandate." They propose the development of a new science, "fisheries management science," which combines the disciplines of fisheries management, fisheries science, and management science. The effect of this, in terms of the division of labour described above, seems to be to expand the role of the group we have called scientists and diminish that of the decision makers. (Very similar views are expressed by Stephenson and Lane 1995.)

Whatever institutional structures are used, and roles assigned, there is clear scope for discussion about what might be called risk management policy. Basson (1993) provides a good example of this by discussing four opposing pairs of attitudes to the role and practice of risk management. The process of deciding which of these attitudes are acceptable in a particular fisheries management forum would clearly be useful in clarifying issues, structures, and procedures. Although scientists have a role in this debate, it is primarily in the realm of decision makers.

Discussion

A common way for scientists to present their advice to fishery managers is as a single number, or sometimes a number with some confidence interval about it. This number is their estimate of the level of catch (or fishing mortality) that is, in some sense, optimal. This form of advice presents difficulties for fishery managers. There is often pressure from other parties to set the catch at a different level, and the advice provides no measure of the consequences of succumbing to that pressure. In principle, the fisheries risk assessment overcomes these difficulties. Rather than presenting a "best" option, it evaluates a range of options by showing the likely consequences of following each of them. Also, it acknowledges and incorporates uncertainty by presenting results in the form of probabilities, or expected values, etc. Further, it attempts to give to those who are charged with the responsibility of making decisions the information they need.

The examples discussed above have focussed on just one of the many types of fisheries management decisions, that concerned with setting the annual harvest rate. However, the same techniques can be applied to other types of decisions, e.g., the value of additional research (Cochrane and Starfield 1992; Powers and Restrepo 1993).

There is some scope to improve fisheries risk assessments. We have discussed the need for some standardization of performance measures and the problem of complexity in presentation. There are also many technical issues concerning model structure, the relative merits of Bayesian and frequentist approaches, etc., that are beyond the scope of this paper. Cordue and Francis (1994) pointed out that little consideration has been given to the accuracy (sometimes alarmingly low) with which performance measures are estimated. However, the greatest need in many fisheries jurisdictions is for an explicit statement of management objectives so that the risk assessment can better support management decisions.

There is great scope to improve the practice of fisheries risk management by making it more formal and less in the category of "negotiations in smoke-filled rooms." This change could produce many benefits. Fisheries would be better managed

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because the available data would be better used, decision-making would be more transparent and better documented, and there would be more strategic planning and fewer ad hoc decisions. The debate on the management of individual stocks would be more constructive (and perhaps less heated) because the focus would shift from annual harvest rate decisions to the criteria on which those decisions should be made. This should give stakeholders a better idea of where the fishery is going. More formal risk management also allows (and even encourages) the construction of standards against which to evaluate, and thus improve, the effectiveness of fisheries management.

We do not wish to minimize the formidable difficulties involved in formalizing the treatment of risk in fisheries management. The greatest of these are the problems of eliciting specific management objectives and establishing acceptable trade-offs between conflicting objectives. As the experience with South African anchovy has shown, these are challenging tasks. They involve decision processes that, while established in the methodological literature, have not yet been used operationally. To us these tasks appear much less tractable, and of much greater importance, than relatively minor issues like establishing appropriate discount rates and the degree of risk aversion of decision makers. It is perhaps important to be realistic about what might be achieved. Decision makers will be not be able to articulate all their trade-offs, and we cannot expect all management objectives to be summarized in a single objective function in all fisheries. However, we do not know of any fisheries where the management could not be improved by being made more formal.

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A rapid method for estimating biomass size spectra of benthic metazoan communities

P.M. Ramsay, S.D. Rundle, M.J. Attrill, M.G. Uttley, P.R. Williams, P.S. Elsmere, and A. Abada

Abstract: The construction of biomass size spectra in aquatic environments suffers from two main problems: (*i*) it is a labour-intensive process, and (*ii*) there is a lack of conformity in methodologies. This study evaluates a technique that produces biomass spectra for benthic metazoans from subsamples stratified using a geometric series of nested sieves. The technique was applied to metazoan communities from three sites, two freshwater and one brackish water, on a small river in southern Britain. Random subsamples of animals from each sieve size were measured using image analysis and showed significant differences in body size, as equivalent spherical diameter (ESD), among all but two sieves. There was no significant difference in ESD among sites. Despite the propensity for long thin animals to pass lengthwise through meshes, sieves contained a consistent range of individual body sizes. Mean individual body size and abundance data for each sieve were used to calculate total biomass. These size spectra were consistent with those from previous studies, and it is hoped that this method will encourage the wider application and more rigorous measurement of biomass size spectra in aquatic ecology.

Résumé : L'établissement des spectres de taille de biomasse dans les environnements aquatiques se heurte à deux problèmes principaux : (*i*) il s'agit d'un processus à forte intensité de main-d'oeuvre et (*ii*) il y a manque d'uniformité dans les méthodologies employées. La présente étude vise à évaluer une technique qui produit des spectres de biomasse pour les métazoaires benthiques à partir de sous-échantillons stratifiés en utilisant des séries géométriques provenant de tamis emboîtés. La technique a été appliquée à des communautés de métazoaires dans trois sites différents, soit deux sites d'eau douce et un site d'eau saumâtre, situés dans une petite rivière dans le sud de la Grande-Bretagne. Des sous-échantillons d'animaux choisis au hasard dans chacune des tailles de tamis ont été mesurés par analyse d'image et on a pu observer des différences statistiquement significatives dans la taille corporelle, exprimée sous forme de diamètre sphérique équivalent (DSE), entre tous les tamis, sauf deux. Il n'y avait pas de différence significative dans les DSE entre les sites. Malgré la tendance à passer à travers les mailles observée chez les animaux filiformes, les tamis contenaient une gamme uniforme de tailles corporelles individuelles. Les données sur l'abondance et sur la taille corporelle individuelles moyennes pour chaque tamis ont été utilisées pour calculer la biomasse totale. Ces spectres de taille étaient en accord avec ceux que l'on a obtenus dans des études antérieures et il est à espérer que cette méthode stimulera une application plus répandue et une mesure plus rigoureuse des spectres de taille de biomasse en écologie aquatique.
[Traduit par la Rédaction]

Introduction

The distribution of biomass among individuals in a community is a fundamental feature of that community that is closely related to its functioning (Strayer 1986) and may reflect basic biotic and abiotic ecosystem processes (Manly 1996; Holling 1992). In aquatic systems, body size distributions are commonly expressed as biomass spectra, following the procedure developed by Sheldon et al. (1972) who expressed population density as total biomass on a logarithmic scale of individual body size. Biomass spectra have since been applied to a wide range of aquatic communities, including benthic communities in marine systems (Schwinghamer 1981, 1985) and standing (Rasmussen 1993; Rodriguez and Magnan 1993; Hanson et al. 1989; Strayer 1986) and running (Bourassa and Morin 1995; Cattaneo 1993; Poff et al. 1993; Morin and Nadon 1991) freshwaters. These spectra have been used to compare taxonomically distinct communities and to facilitate the development of general theories of benthic community function (Strayer 1991; Cattaneo 1993; Schwinghamer 1981). As the shape of size spectra appear to be very conservative in pristine habitats they may have another important use in assessing the impact of environmental perturbation (Warwick 1993).

Despite their high ecological value in aquatic research, the use of size spectra has suffered from two main problems. Firstly, their construction involves highly labour-intensive procedures, especially when the full spectrum of metazoan body size is considered and the measurement of many thousands of individuals within the meiofaunal size range is required (e.g., Bourassa and Morin 1995). Secondly, a wide variety of subsampling, body measurement, and conversion factors have been used to estimate biomass, and a variety of categories and scales have been used to plot biomass spectra (Table 1). This inconsistency in methodologies leads to difficulties when comparing size spectra from different systems, a process that often involves recalculation from raw data.

This paper presents a method that allows more rapid biomass estimations and provides an ecologically meaningful

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P.M. Ramsay,[1] S.D. Rundle, M.J. Attrill, M.G. Uttley, P.R. Williams, P.S. Elsmere, and A. Abada. Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, U.K.

[1] Author to whom all correspondence should be addressed. e-mail: pramsay@plymouth.ac.uk

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Table 1. Methodologies used in the construction of size spectra in a selection of publications that consider the entire metazoan community.

Source	Subsampling	Measurements	Conversion factors	Size categories
Poff et al. (1993)	Macrofauna: used, but not specified	Macrofauna: body length	Power equations	Log_{10} intervals of biomass
	Meiofauna: used, but not specified	Meiofauna: none	Average biomass from literature	
Strayer (1986)	Not specified, but "usually at least 10 of each species"	Linear body dimensions	Power equations and simple geometric shapes	Half an order of magnitude on a log_{10} scale
Morin and Nadon (1991); Bourassa and Morin (1985)	Macrofauna: not used	Body length	Biomass as DM assumed to be cube of body length	Log_2 intervals of biomass
	Meiofauna: subsample of 50–200 animals measured			
Schwinghamer (1981)	Macrofauna: not specified, if used	Volume displacement of individuals or groups of similarly sized individuals of a taxonomic group	Assumed volume equals mass in conversion to ESD	Log_2 intervals of ESD
	Meiofauna: not specified, if used	Linear body dimensions	Simple geometric shapes	
Cattaneo (1993)	Used, but not specified	Body length	Power equations	Log_2 intervals of ESD

Note: In each case, details are given of subsampling approach (often used but not specified), the measurements taken from animals and the conversion factors used to translate these measurements into size and biomass values. The size categories used to plot the size spectra are also shown. DM, dry mass.

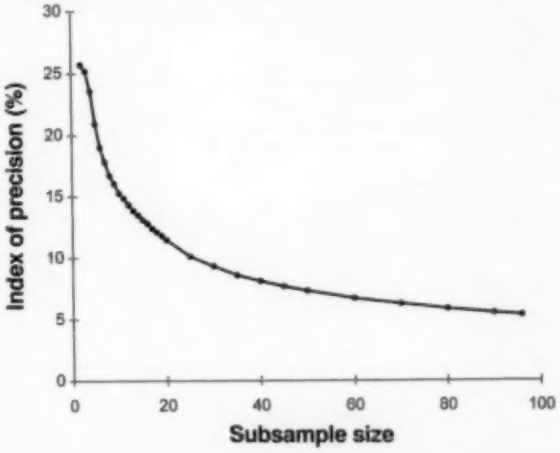
Table 2. Physical and chemical properties of the three sites on the River Yealm, southern Britain.

	Upper freshwater	Lower freshwater	Brackish
Width (m)	8.8 (0.3)	11.8 (0.7)	~100
Distance from source (km)	8.5	13.5	14.4
Conductivity ($\mu S \cdot cm^{-1}$)	147 (8)	209 (18)	2094 (966)
pH	7.3 (0.1)	7.7 (0.1)	8.1 (0.2)

Note: Widths are means calculated from 10 random measurements, and chemical data are means from seasonal samples, with SEs shown in parentheses.

scale of size categories for plotting biomass size spectra. The technique is based on a procedure developed by Edgar (1990) for assessing macrofaunal productivity and biomass. It involves passing animals through a series of nested sieves, calculating a standard mean individual biomass for each mesh-size interval, and then using these standard values to calculate biomass spectra from abundance data. One of the most important considerations when constructing size spectra is that the scale along the body-size axis is ecologically meaningful with a resolution of data points fine enough to distinguish peaks and troughs in the data. Size spectra are traditionally plotted using logarithmic axes, though there has been little consistency in the choice of scale used (Table 1). It has been suggested that the best scale for individual body size categories is a two times geometric series (Warwick 1984). This scale corresponds with Hutchinson's size ratios for explaining niche differentiation among coexisting species (Hutchinson 1959), and is perhaps of most biological and ecological relevance. The proposed technique uses the Wentworth mesh-size series, with a doubling of mesh area at each stage,

Fig. 1. The relationship between precision and subsample size. The biomass of 96 animals was estimated according to the method described in the text. From this pool of animals, 100 simulated random subsamples were generated for each of 30 subsample sizes and an index of precision was calculated as the ratio of SE to arithmetic mean of the biomass estimations.



which, potentially, should relate to the Hutchinsonian separation.

The technique was applied to benthic metazoan samples from three sites (two freshwater and one brackish water) on the same river. Several questions concerning the suitability of the technique are addressed. Firstly, does the range of sieves provide a useful and ecologically meaningful separation of

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Fig. 2. The mean size (ESD) of invertebrates trapped in the 10 sieves (n = 120) with 95% confidence limits. Means sharing a letter were not significantly different (Student–Newman–Keuls multiple-range test).

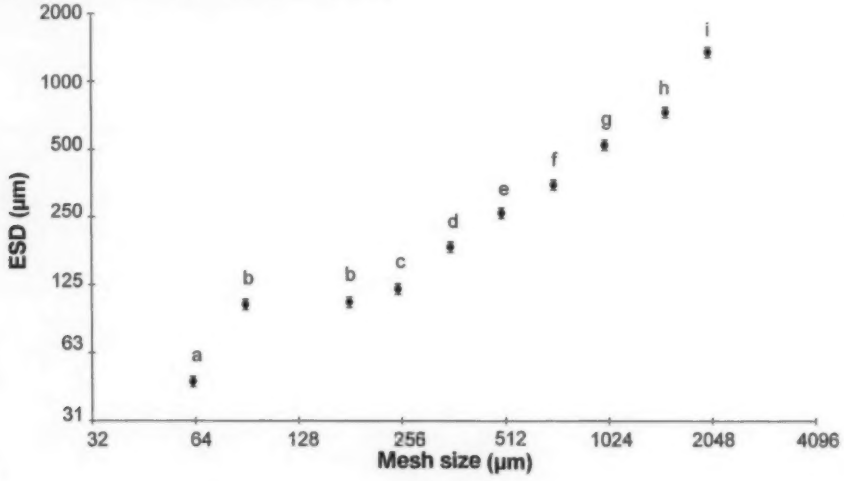

Table 3. The number of individuals for which length–mass relationships were used (power equation) and for which simple geometric shape categories were applied, ratio of body length to mesh size for each sieve, and mean individual ESD values (mm) for each sieve.

	Mesh size (µm)										
Power equation	2000	1500	1000	710	500	355	250	180	90	63	Total
No. of individuals											
Power equation	97	99	109	101	85	85	103	109	102	3	893
Cylinder	23	21	11	19	35	16	11	2	8	117	263
Half cylinder						10					10
Sphere						9	6	9	10		34
Total	120	120	120	120	120	120	120	120	120	120	1200
Ratio of body length to mesh size											
Power equation	3.4	2.4	2.6	2.3	2.4	2.2	1.0	0.7	0.7	6.5	1.9
Cylinder	6.3	4.3	4.9	5.0	4.7	4.6	3.5	4.5	7.9	10.9	7.7
Half cylinder						2.1					2.1
Sphere						1.3	1.5	1.3	2.4		1.7
Overall	3.9	2.7	2.8	2.8	3.1	2.4	1.2	0.8	1.3	10.8	3.2
Mean individual ESD values (mm)											
Power equation	1.38	0.72	0.52	0.33	0.23	0.18	0.11	0.10	0.11	0.08	0.41
Cylinder	1.18	0.73	0.55	0.44	0.32	0.20	0.15	0.13	0.08	0.05	0.30
Half cylinder						0.17					0.17
Sphere						0.22	0.13	0.09	0.08		0.13
Overall	1.34	0.72	0.52	0.35	0.26	0.18	0.12	0.10	0.10	0.05	0.37

organisms across the biomass size spectrum? Secondly, what is the role of body size and shape in determining the fate of individual organisms in the nested sieves, and how does this influence the estimates of size and biomass? Finally, are the estimates obtained using this technique consistent with previously published biomass size spectra?

Materials and methods

Sampling and sample processing

Three sites were sampled on the Yealm, a small river approximately 5 km east of Plymouth in southern Britain. It flows 24 km from Dartmoor National Park (peaty gleys and podzols on granite) through agricultural land (Devonian slate) to the English Channel. Two sites were freshwater and the third brackish water (Table 2).

Four replicate samples of benthic invertebrates were collected from stony riffle zones at each site with a Surber sampler (0.1 m^2; 63-µm mesh) in July 1994 and preserved in the field in formalin. This procedure is appropriate for sampling metazoans in both the macrofaunal and meiofaunal size range (Hellawell 1986; Rundle 1990). In the laboratory, samples were sorted by washing animals through a series of 10 brass-frame laboratory test sieves with steel meshes graded on the $\sqrt{2}$ Wentworth scale (Buchanan 1984): 2000, 1500,

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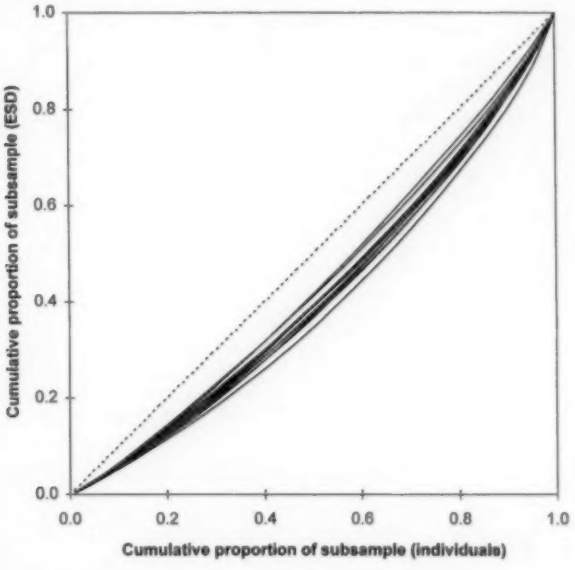
Fig. 3. Lorenz curves for the size (ESD) of subsampled organisms trapped in each of the 10 sieve sizes. The isometric line (broken) denotes a situation in which all individuals in a sieve are exactly the same size.



1000, 710, 500, 355, 250, 180, 90, and 63 µm. In this series, each step represents a halving in the area of the mesh aperture. The first stage in the procedure involved separating the macrofauna from the meiofauna, by washing samples through 500-and 63-µm sieves, respectively. The macrofauna were then separated from mineral material by elutriation, prior to washing through the 2000–500 µm sieves. Meiofauna were separated from fine sediments using the ludox flotation method (McIntyre and Warwick 1984) and then washed through the 355–63 µm sieves. The number of individuals in major taxonomic groups (see Appendix) in each mesh size replicate was then counted; replicates with high numbers were subsampled, but at least 100 organisms from each were used to estimate total abundances.

Biomass estimations

To determine an acceptable number of measurements for the estimation of biomass from each sieve, 96 animals were taken at random from one sieve (the 500-µm sieve from a replicate sample of the lower freshwater site; both sieve and sample were chosen randomly) and their biomass estimated as described below. Simulated random subsampling of these data provided 100 estimations of mean individual biomass for a series of subsample sizes. In each case, an index of precision was calculated as the ratio of the standard error to the mean (Elliott 1977), and the results are summarized in Fig. 1. Accepting a standard error at or below 20% of the mean ("a reasonable error in most bottom samples;" Elliott 1977), we chose to measure 10 animals from each sieve sample (14% precision for the sample in question), though more measurements could be used to provide greater precision.

Animals were measured for biomass estimations using a Quantimet 570 Image Analyser attached to a Kyowa Trinocular stereozoom microscope. For those taxa with published length–mass relationships (Pearre (1980) for Copepoda; Smock (1980) for adult Coleoptera and Hemiptera; Meyer (1989) for Amphipoda, Coleoptera larvae, Chironomidae, non-Chironomid Diptera, Ephemeroptera, Plecoptera, Trichoptera, and Tricladida) biomass was calculated from the power equation: $m = al^b$, where m is the dry mass of the organism, l is the length, and a and b are constants for each taxon.

For taxa without published length–mass relationships, organisms were approximated to simple geometric shapes (Winberg and Duncan 1971). Collembola, Decapoda, Diptera pupae, Nematoda, Oligochaeta, Ostracoda, Phyllodocida, Rotifera, and Tardigrada were approximated to cylinders. Cladocera, Hirudinea, and Isopoda were approximated to half cylinders. Hydracarina were approximated to spheres. Animal volumes were converted to dry weights assuming a specific gravity of 1.05 and a dry- to wet-weight ratio of 0.15 (Strayer 1986).

Total biomass for each sieve size was estimated by multiplying the average biomass of the randomly selected individuals by the total number of individuals found in that sieve.

Size was expressed as an equivalent spherical diameter (ESD), to standardize for the variety of body shapes, calculated using the following equation (Schwinghamer 1981):

$$\mathrm{ESD} = 2\left(\frac{3m}{4\pi d}\right)^{1/3}$$

where m is the wet mass of the organism and d is its density (assumed to be 1.05 for all organisms at all three sites; Strayer 1986).

Size inequality can be presented graphically as a Lorenz curve (Lorenz 1905), originally used by economists to represent the distribution of wealth or income in societies. The animals caught in a particular sieve are first ranked according to size. Then, starting with the smallest animal and ending with the largest, the cumulative proportion of individuals is plotted against the cumulative proportion of total ESD in the sieve. If all of the individuals in a sample were exactly the same size, the result would be a straight line from the origin to the opposite corner, as each animal added contributes exactly the same ESD. Any inequality in size results in a curve below this isometric line. The proportion of the area below the isometric line between that line and the sample curve is known as the Gini coefficient, G (Gini 1912), and provides a quantification of size inequality within a sample (Weiner and Solbrig 1984), allowing the contents of different sieves to be compared on this basis. In essence, this coefficient is a measure of relative precision, similar in many ways to the coefficient of variation, but less sensitive to outlying values (Bendel et al 1989). Gini coefficients were calculated for pooled replicate samples for each sieve from each site ($n = 40$). The Gini coefficient has a minimum value of 0 (no inequality) and a theoretical maximum of 1 (all the individuals but one in an infinite population have a value of 0). The coefficient can be obtained directly from the data by calculating the arithmetic average of the absolute differences between all pairs of individuals and dividing by two. Applying a correction to give unbiased values, G', the equation is

$$G' = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}|x_i - x_j|}{2n^2\bar{X}} \cdot \frac{n}{n-1}$$

where x_i and x_j represent all possible pairs of individual ESD values, n is the number of individuals, and $\bar{X}$ is the mean of the ESD values.

Results

Harpacticoid copepods were the most abundant taxon across sites, accounting for 28–50% of individuals (Appendix). Overall, however, the taxonomic composition differed substantially between the freshwater and brackish water sites: Chironomidae, Ephemeroptera, and Oligochaeta were among the most abundant taxa in the freshwater samples, whereas Nematoda, Ostracoda, Hydracarina, and Cyclopoida were numerically dominant in brackish water.

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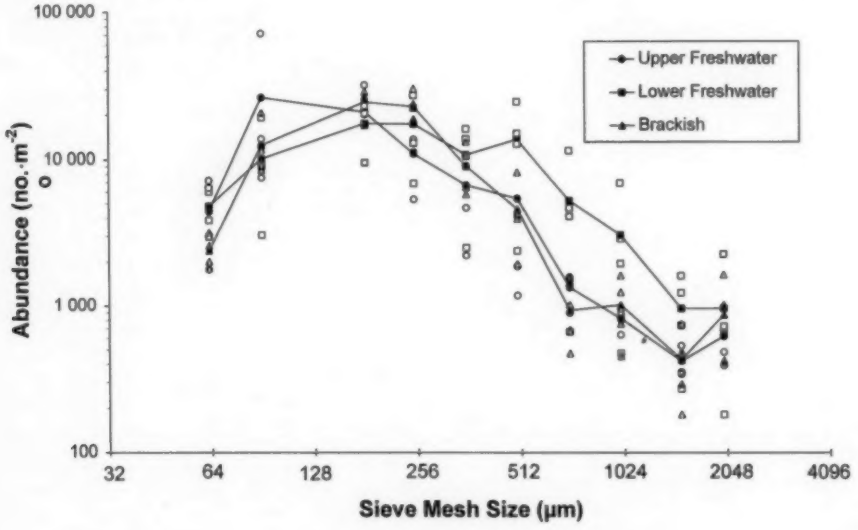
Table 4. Unbiased Gini coefficients (G') of ESD values for each sieve for pooled replicates from each site.

	Mesh size (μm)									
	2000	1500	1000	710	500	355	250	180	90	63
Upper freshwater	0.20	0.18	0.22	0.16	0.14	0.19	0.21	0.12	0.13	0.15
Lower freshwater	0.21	0.19	0.17	0.19	0.11	0.14	0.12	0.13	0.10	0.18
Brackish	0.20	0.18	0.15	0.19	0.17	0.12	0.20	0.14	0.14	0.14
Overall	0.22	0.19	0.18	0.18	0.16	0.22	0.18		0.15	0.16

Note: The overall value was calculated by pooling all samples.

Fig 4. The abundance of invertebrates in the 10 sieves. Mean values for each site (solid symbols) were derived from four replicate samples (open symbols).



An analysis of variance (ANOVA) on log-transformed ESD values for individual organisms indicated no significant differences in the size of animals measured at the three sites. There was, however, a highly significant difference among sieve sizes ($p < 0.0001$), and with the exception of the 90- and 180-μm sieves, the size of organisms trapped in any one sieve size was significantly different from all other sieve sizes (Student–Newman–Keuls multiple-range test) (Fig. 2). There was also a significant interaction between site and sieve sizes resulting from among-site differences in the separation of data points along the body size axis, although this had little effect on the construction of biomass spectra (see below).

Most (72%) of the animals used in calculating mean ESD values were those for which power equations were available (Table 3). Of the remainder, 22% were assumed to be cylinders and only 4% were classified as another body form. To assess whether body shape influenced biomass calculations the mean length of different body shapes was expressed as a ratio of mesh size (Table 3). Cylinder-shaped animals were, on average, about eight times longer than the mesh size, whereas animals whose biomass was calculated from power equations were approximately twice the length of the mesh size. Despite these differences, animals with different shapes had similar mean individual ESD values for any given mesh size (Table 3). In other words, the greater length of a cylindrical organism was balanced by a lesser body width. This was confirmed by the Lorenz curves (Fig. 3) and Gini coefficients (Table 4), which were similar for each sieve size; the latter had values between 0.10 and 0.22, suggesting that the range of sizes within each sieve was consistent across the mesh sizes.

The relatively consistent size distribution within each sieve was also reflected in the precision of mean biomass estimates for the sieves: the index of precision (described earlier) was below 20% for all sample means, below 15% for 118 of the 120 samples, and was 10% or less in over 90 of the samples.

Maximum abundances occurred in the 90-μm sieve at all sites, i.e., within the small meiofaunal range of body size, with a trough in all sites in the 1500-μm sieve (Fig. 4). Variation in abundance was high both among and within sites, and this pattern is mirrored in the scatter of replicate biomass values (Fig. 5*a*), demonstrating that the main determinant of total biomass for a particular body size is the abundance of animals rather than their individual body size. Despite high variability among replicates, mean biomass spectra showed a very similar pattern between sites (Fig. 5*b*).

Discussion

The main aim of this paper was to evaluate a new technique for the construction of biomass spectra for benthic communities.

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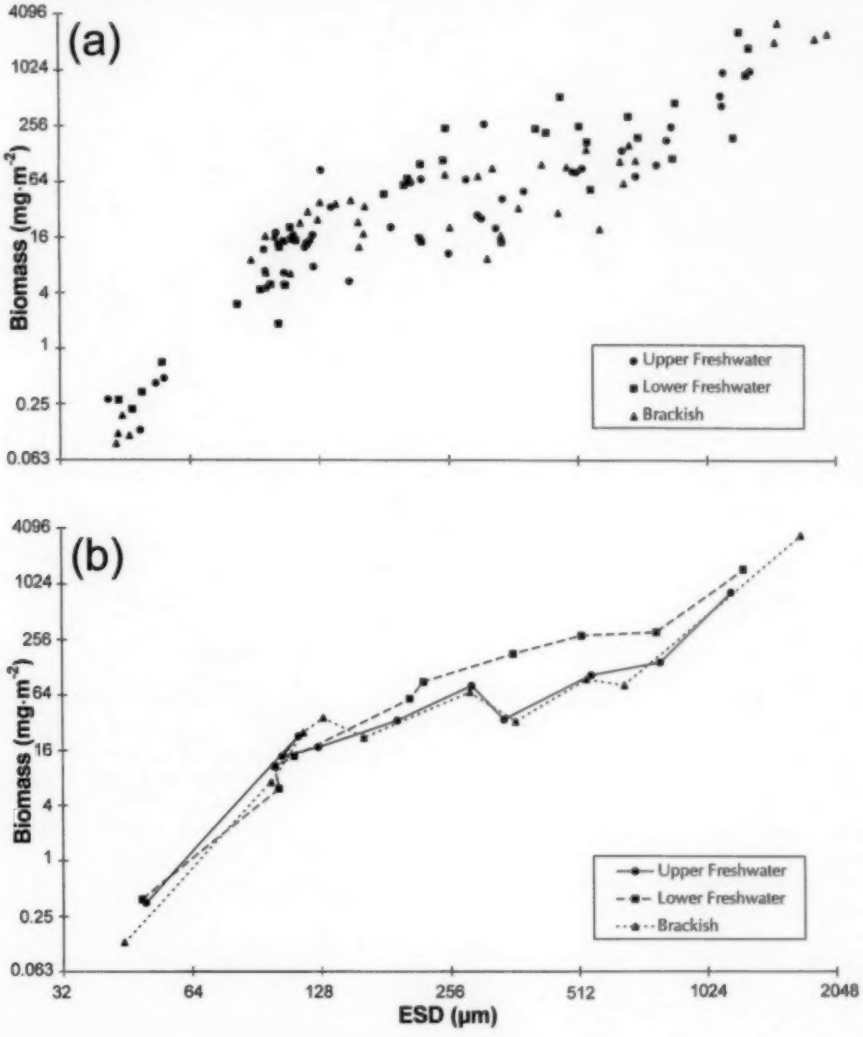
Fig. 5. Biomass size spectra for benthic metazoans from the River Yealm: (*a*) scatter of values from all 12 replicates; (*b*) mean biomass size spectra for each site (pooled replicate data).



This technique reduces the amount of time taken in producing biomass size spectra, with measurements for relatively few organisms from each replicate sieve required to gain a reasonable precision. The method also provides a standard, ecologically meaningful scale for the categorization of body size in size spectra; the Wentworth mesh size series provides relatively evenly spaced data points with a fine resolution along the body size axis.

One potential source of error in sieving methods is the influence of an animal's shape on its propensity to pass through a sieve. For example, Edgar (1990) found that the mean weight of animals in some sieves was increased by the presence of a few individuals from cylindrical taxa (e.g., polychaete worms) whose shape meant that they passed through sieves where other taxa of similar biomass were caught. He also found that some of these longer taxa became coiled around the mesh wires, reducing the mean animal weight in coarse sieves. He concluded that studies using sieving methods for estimations of biomass or production were not valid where a high proportion of the taxa were long and flexible. In contrast, our study suggests that although, on average, long thin taxa were longer than animals with other body forms collected in the same sieve, their length was balanced by their relatively smaller width to give similar individual body volumes and biomass. In addition, the ESD values for each sieve showed similar Lorenz curves and Gini coefficients, which suggests similarity in relative body volume range throughout the sieves.

Therefore, at least for the samples taken in our study, body shape does not appear to influence the estimates of volume and biomass within each sieve. However, it is not clear whether this is can be relied upon for samples taken elsewhere or at other times. The evidence from marine systems suggests that

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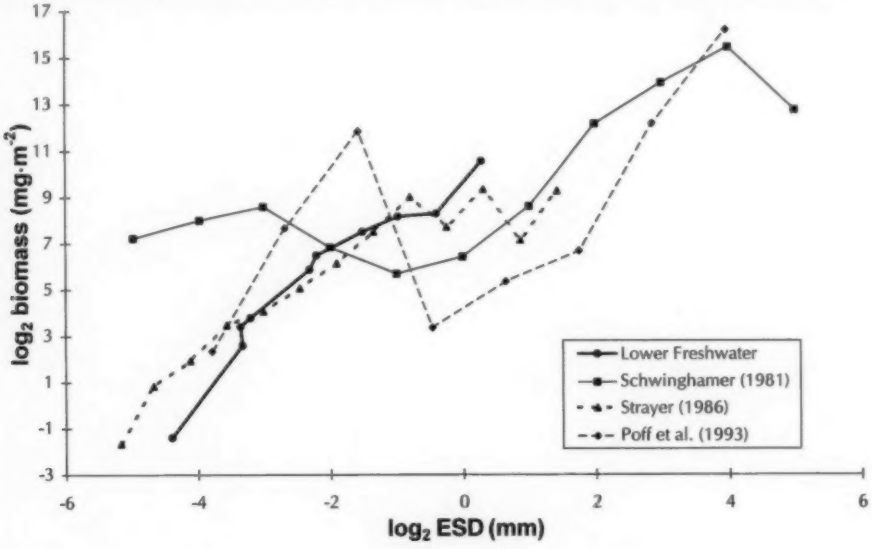
Fig. 6. Mean biomass size spectrum from the lower freshwater site compared with spectra from other published studies.



different body shapes of the same biomass may be caught in different sieves, particularly polychaetes, oligochaetes, and nematodes (Edgar 1990; Bachelet 1990). It is advisable, therefore, that cylindrical animals are measured and counted separately from those with other body shapes in a sieve.

To assess whether our technique produced results consistent with those for other aquatic systems, the biomass spectrum for the lower freshwater site is plotted against spectra from a range of systems in Fig. 6. For clarity we have compared only with those other studies that included the full metazoan size range. The general pattern of our data is most similar to that of Strayer (1986) for Mirror Lake in New Hampshire and shows no evidence of the bimodality of Schwinghamer (1985) and Poff et al. (1993) (intertidal and riverine benthos, respectively).

A major difference between our data and those from the other studies is the absence of large metazoans in our samples. This can be explained by the fact that most of the biomass at large body sizes in the freshwater studies of Poff et al. (1993) and Strayer (1986) belonged to large bivalves, taxa which were absent in our freshwater sites. It is also possible that the scale of sampling undertaken at the estuarine site was insufficient to sample large body sizes and more replication might be required to reduce the high variances associated with small-scale macrofaunal samples when the distribution of these organisms is highly patchy.

In conclusion, the main advantage with our technique is that it saves considerable time with the measurements for biomass determination, therefore, allowing for improvements in other areas of the sampling programme. In particular, it enables increased sample replication and thus increases the reliability of data. The importance of metazoan abundances in calculating biomass in our study (compare Figs. 5*a* and 5*b*) stresses the value of obtaining good quantitative abundance data. Time is better spent processing more replicate samples rather than measuring more animals from fewer samples where assumptions are already substantial, such as the use of equations for estimating the body size of animals from different geographical locations (Meyer 1989) and the assumption that all organisms have the same dry- to wet-weight ratio and density.

This study is the first in an ongoing project in our laboratory on body-size spectra in aquatic communities. With more data from a greater variety of aquatic habitats it may be possible to determine reliable estimates for individual body size or biomass for sieves and to apply these standard values across all sites. This technique also lends itself to automation of measurement, consistent with Sheldon's original approach (Sheldon et al. 1972).

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Appendix. The number of animals of each taxon present in the samples. For each site, and overall, the number of animals in the Surber samples are shown, together with the number selected at random as subsamples from the sieves. The data for each site are pooled from four replicate samples.

	Upper freshwater site		Lower freshwater site		Brackish site		Total	
Order	Surber samples	Sieve subsamples	Surber samples	Sieve subsamples	Surber samples	Sieve subsamples	Surber samples	Sieve subsamples
Copepoda	15 764	85	9 660	59	17 450	126	42 874	270
Diptera (Chironomidae)	3 811	82	7 535	102	211	2	11 557	186
Oligochaeta	5 300	76	3 315	28	749	18	9 364	122
Ephemeroptera	1 341	52	6 729	69	16		8 086	121
Nematoda	2 060	41	948	39	3 540	42	6 548	127
Ostracoda	714		1 080		5 110	3	6 904	3
Hydracarina	372	8	1 015	11	2 136	15	3 523	34
Rotifera	584		1 076		248		1 908	
Amphipoda			1		1 504	170	1 505	170
Trichoptera	119	13	708	14			827	27
Diptera (larvae)	238	9	429	11			667	20
Plecoptera	281	20	334	42		1	615	63
Cladocera	314		184				498	
Collembola	2				332		334	
Coleoptera (adults)	35	1	209	6			244	7
Coleoptera (larvae)	19	9	181	10			200	19
Tricladida	26	1	166	9			192	10
Hemiptera	70		13		1		84	
Phyllodocida					79	6	79	6
Isopoda		2			50	5	50	7
Decapoda					39	6	39	6
Prosobranchiata	2		40		1		43	
Diptera (adults)	1	1	18				19	1
Tardigrada			8		1	1	9	1
Pulmonata	2		1				3	
Hirudinea	2						2	
Totals	31 056	400	33 650	400	31 467	400	96 172	1200

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Demersal fish biomass size spectra on the Scotian Shelf, Canada: species replacement at the shelfwide scale

Daniel E. Duplisea, Stephen R. Kerr, and Lloyd M. Dickie

Abstract: Three parameters were derived from annual biomass size spectra constructed for a 22-year time series of demersal fish data for the entire Scotian Shelf: (*i*) total sampled biomass density, (*ii*) modal body weight, and (*iii*) the shape of the various biomass size spectra. Temporal variability of these parameters was compared among six species groups and between the species groups and their total. Species-group biomass varied more over the time series than did the total biomass, indicating the presence of a system factor. Conversely, modal body weight was as variable, and sometimes more variable, for the total than it was within species groups, indicating variability mainly at the species level. The shape of the biomass size spectrum showed annual differences in variability owing mainly to contributions of species groups. This variability was higher than variability in the combined species totals. We conclude that total biomass is limited at the combined species (systemwide) level because it depends on energy input to the system. Variability is greater at the species group (subsystem) level because the multiage species-group biomasses do not equally reflect a given energy limitation. Neither modal body weight nor the shape of the biomass spectrum is sensitive to the degree of taxonomic aggregation, suggesting that both measures are independent of energy input (primary production). We found no evidence of consistent species replacement trends on the shelfwide scale and conclude that diffuse replacement best describes species succession on the Scotian Shelf as a whole.

Résumé : Trois paramètres ont été dérivés des spectres annuels de taille de la biomasse reconstitués à partir d'une série chronologique de 22 ans sur les poissons démersaux de l'ensemble de la plate-forme Néo-Écossaise : (*i*) la densité de biomasse échantillonnée totale, (*ii*) le poids corporel modal et (*iii*) la forme des divers spectres de taille de biomasse. La variabilité temporelle de ces paramètres a été comparée dans six groupes d'espèces et entre ces groupes d'espèces et leur total. La biomasse des groupes d'espèces a varié davantage au cours de la série chronologique que la biomasse totale, ce qui indique la présence d'un facteur de système. À l'opposé, le poids corporel modal était tout aussi variable, et parfois même plus variable, pour la totalité qu'il ne l'était au sein des groupes d'espèces, ce qui indique une variabilité intervenant principalement au niveau de l'espèce. La forme du spectre de taille de la biomasse a révélé des différences annuelles dans la variabilité attribuables principalement aux contributions des groupes d'espèces. Cette variabilité était plus prononcée que la variabilité observée dans les totaux combinés des espèces. Nous concluons que la biomasse totale est limitée au niveau des espèces combinées (à l'échelle du système) parce qu'elle dépend de l'apport d'énergie dans le système. La variabilité est plus élevée au niveau du groupe d'espèces (sous-système) parce que les biomasses des groupes d'espèces multi-âges ne reflètent pas également une limite énergétique donnée. Ni le poids corporel modal ni la forme du spectre de biomasse ne sont sensibles aux degrés d'agrégation taxinomique, ce qui indiquerait que les deux mesures sont indépendantes de l'apport d'énergie (production primaire). Nous n'avons décelé aucune indication de tendance uniforme de remplacement des espèces à l'échelle de la plate-forme, et concluons que le remplacement diffus est le mécanisme qui décrit le mieux la succession des espèces dans la plate-forme Néo-Écossaise dans son ensemble.
[Traduit par la Rédaction]

Introduction

A biomass size spectrum is the distribution of biomass per unit area as a function of body size in an ecosystem (the Sheldon spectrum; Schwinghamer 1981). Biomass size spectrum models indicate that aquatic size distributions should be characteristic in form and temporally stable, in the absence of major disturbances (Sheldon et al. 1972; Kerr 1974; Thiebaux and Dickie 1992, 1993). Empirical work has confirmed the expected pattern, and its stability (Sheldon et al. 1972, 1973; Pope et al. 1988; Sprules and Goyke 1994; Duplisea and Kerr 1995; Macpherson and Gordoa 1996).

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D.E. Duplisea.[1] Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada, and Department of System Ecology, Stockholm University, S-106 91 Stockholm, Sweden.
S.R. Kerr. Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.
L.M. Dickie. Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1002, Dartmouth, NS B2Y 4A2, Canada.

[1] Author to whom all correspondence should be sent at the following address: Department of System Ecology, Stockholm University, S-106 91 Stockholm, Sweden. e-mail: danield@system.ecology.su.se

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Recent analyses of aquatic biomass size spectra have made two valid simplifying assumptions. The first is that metabolic dissipation by aquatic organisms increases as a function of body mass, with a power function index that for many aquatic poikilotherms is typically in the order of 0.75 (Peters 1983). The second is that density distributions are a function of size-structured predation, where large eat small in a preferred body size ratio (Hansen et al. 1994). In the context of total system energy transfer, about 90% of a wide variety of predator–prey interactions involve large eating small (Cohen et al. 1993), and this percentage may be greater for aquatic communities, considering the constraints that are placed on predation events (Sheldon et al. 1977). Accordingly, we have studied the relative densities, body sizes, and distributional patterns of the fish biomass spectra obtained from trawl sampling.

Aquatic biomass size spectra are expected to exhibit variability at least two levels of scaling, which reflect the effects of different levels of organisation (Dickie et al. 1987). A primary or physiological level of scaling for the whole spectrum appears as a straight line of slight negative slope (Platt and Denman 1977, 1978) or a broad parabola (Thiebaux and Dickie 1992, 1993). Variance about the primary slope or parabola indicates that there are periodic departures of biomass corresponding to trophic positions (Boudreau et al. 1991; Sprules et al. 1991; Boudreau and Dickie 1992). These secondary positions or domes result from ecological interactions associated with predation processes. In addition, the model developed by Thiebaux and Dickie (1993) suggests that the same scaling considerations can be applied to the parabolic dome describing a single trophic position. That is, predation effects should give rise to secondary biomass domes within it. Recent studies applied the Thiebaux and Dickie (1992, 1993) model (the T–D model) to data for the Lakes Michigan and Ontario ecosystems (Sprules and Goyke 1994; Sprules and Stockwell 1995). Data corresponded with the model at both levels, that is, for the trophic domes from phytoplankton to fish, and within these trophic domes.

Analysing a 22-year series of trawl survey data, Duplisea and Kerr (1995) showed that the T–D model provides an accurate description of the biomass dome of the demersal fishes on the Scotian Shelf of Atlantic Canada. We deal here more extensively with attributes of this trophic position, with particular interest in how it is reflected in the species composition of the demersal and benthic fish community. Generally speaking, biomass spectrum analysis has not dealt directly with the details of species composition. However, fisheries management, because of its market orientation as well as its origins in the times when little was understood of fisheries production systems, remains rooted in the species concept. It is therefore useful to examine some taxonomic ramifications of the biomass spectrum, which is emerging as a powerful generalization of the community production processes underlying fisheries yields.

Pope et al. (1988) examined the relative abundance and length distribution of various species groups of demersal fishes in the North Sea and on Georges Bank over extended periods of trawl sampling. For Georges Bank, which is near the Scotian Shelf, they found that the species composition changed from a dominance of gadoids, valued by the commercial fishery, to a dominance of dogfish sharks, not desirable to the fishery. In the North Sea, similar species replacement was not obvious.

From our recent examination of the 22-year record of trawl survey data for the Scotian Shelf (Duplisea and Kerr 1995), the groundfish community has exhibited a stable biomass spectrum (Fig. 1), despite major changes in the densities of individual species that have occurred over this time (Angel et al. 1994). Obviously, overall stability of the biomass spectrum but internal (species) variability must entail some form of species replacement. In the following we enquire whether these compensatory changes result from identifiable shifts in the abundances of taxonomic identities (direct species replacement), as indicated by Pope et al. (1988) for Georges Bank, or whether the overall stability of the Scotian Shelf demersal fish biomass dome has resulted from numerous, less obvious, changes in species abundances (diffuse species replacement) as suggested for the North Sea fishery.

Methods

Data from the Canadian Department of Fisheries and Oceans (DFO) Summer Groundfish Survey (for details of the survey procedures used, see Doubleday 1981; Halliday and Koeller 1981) from 1970 to 1991 on the Scotian Shelf were organized into biomass density size spectra. These spectra were obtained by converting length–frequency distributions of individual fish species sampled by standardized net hauls into biomass-at-weight distributions, using length–weight regressions for each species. Species biomass-at-weight distributions, expressed as weight-at-size, per standard trawl haul are therefore relative biomass density estimates that can be combined according to broad taxonomic (Scott and Scott 1988) and (or) functional groupings. A standard haul of a 12-m trawl net for 1.75 nautical miles (1 nautical mile = 1.852 km) is the equivalent of sampling about 39 000 m^2.

We have compiled spectra for (1) gadoids (–SH, excluding silver hake); (2) silver hake (*Merluccius bilinearis*); (3) dogfish (*Squalus acanthias*); (4) flounders (Pleuronectidae); (5) skates (Rajidae); and (6) "others", consisting of a number of smaller species that did not fit into any of the previous categories. These groupings are similar to those used by Pope et al. (1988) and de Aracama (1992). For purposes of illustration we have combined data into two larger species subgroups: (7) gadoids (+SH) a more general gadoid group including silver hake and (8) benthic fishes, a group consisting of flounders and skates combined. We consider 7 and 8 ecological groupings and use them for clarity in Fig. 2.

To calculate an overall biomass density for the Scotian Shelf, each species-group size distribution was subjected to area weighted averaging, the assigned weight being proportional to the area of the depth stratum where the randomly positioned survey haul was taken (see Doubleday 1981 for a description of depth strata organization on the Scotian Shelf). However, because annual average density varied by a factor of two over the period, size-classes within the biomass size spectra are presented in Fig. 2 as proportions of the total annual biomass density. To facilitate inter-year and -system comparison, these are plotted against $\log_2$ groupings of body weight. Log_2 body weight groupings are used here for consistency with previous biomass size spectrum studies (Sheldon et al. 1972; Platt and Denman 1977, 1978; Schwinghamer 1981; Rodríquez and Magnan 1993). The $\log_2$ class value represents the smallest body weight for the class, e.g., weight-class 7 represents all fish within the body weight range of 2^7 to 2^8 g or 128–256 g. Proportional biomass can be back-calculated to biomass density by multiplying the percentage in each weight-class by total biomass (Table 1). All statistical analyses are based on biomass values, not proportional values.

The coefficient of variation (CV = standard deviation/mean) is used to assess variability in the time series of spectra. Temporal variability of three measures is calculated: (*i*) total sampled biomass density ΣB

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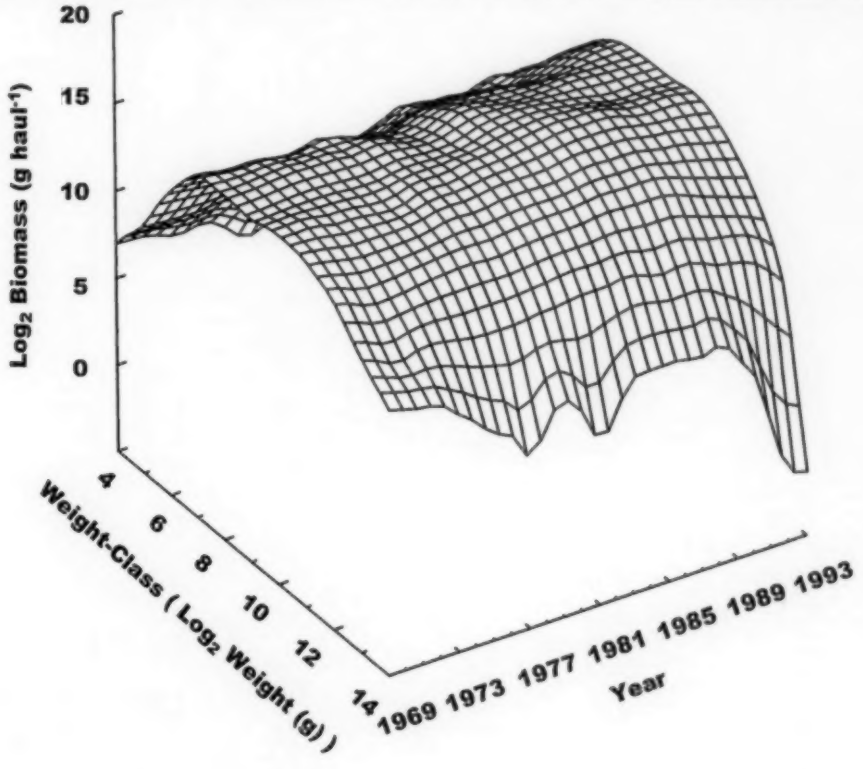
Fig. 1. Demersal fish biomass size-spectrum on the Scotian Shelf from 1970 to 1991. Surface interpolated with distance-weighted least squares.



of each species-group and the total, (*ii*) modal body weight ($\bar{W}$) of each species group and the total, and (*iii*) the index of dispersion (I) of the spectrum of each species group and the total spectrum. These were calculated as

$$(1) \quad \sum B = \sum_{W=4}^{14} B_W$$

$$(2) \quad \bar{W} = \frac{\sum_{W=4}^{14} N_W \cdot W}{\sum N}$$

where

$$\sum N = \sum_{W=4}^{14} N_W$$

and

$$(3) \quad I = \frac{\bar{W}}{s^2}$$

where

$$s^2 = \left[\frac{\sum_{W=4}^{14} ((\bar{W} - W)^2 \cdot N_W)}{\sum N} \right]^2$$

where W is the weight-class; B_W is the biomass density of fish in weight-class W; N_W is the numerical density of fish in weight-class W; and s^2 is the variance. N_W can be approximated by dividing B_W by W.

Biomass density is calculated over the observed body size range (total biomass/total number). The exact value thus depends on the bounded body size; that is, the values for these non-normalized plots are only comparable between studies that have chosen the same interval boundaries (not the same as the normalized plots of Platt and Denman 1977, 1978). I is a measure of the shape of a distribution (Krebs 1989), where $I = 1$ for a Poisson distribution.

Results

Analysis of size spectra and biomass contribution

The general gadoid group (7), gadoids (+SH), usually made up the greatest percentage (>50%) of class-specific biomass in the larger weight-classes (Fig. 2). These formed a broad biomass dome of seven or eight weight-classes, from logs 7 to 13, around a mode that was about 15% of total biomass in weight-class 10 (1–2 kg). Dogfish were present only in weight-classes larger than 8 (>250 g). The dogfish biomass mode was also in weight-class 10 and was, on average, about 10% of total biomass (1–2 kg). However, its biomass at the mode varied considerably among years (0.6% in 1978 to 23% in 1971). Dogfish and gadoids (+SH) together were almost sole constituents of the larger weight-classes. Biomass contributed by benthic fish was spread throughout almost the entire size range with a mode of about 2% of total in weight-class 8 or 9

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Fig. 2. Proportional biomass size-spectra of total demersal fish biomass on the Scotian Shelf from 1970 to 1991 partitioned among four species groups.

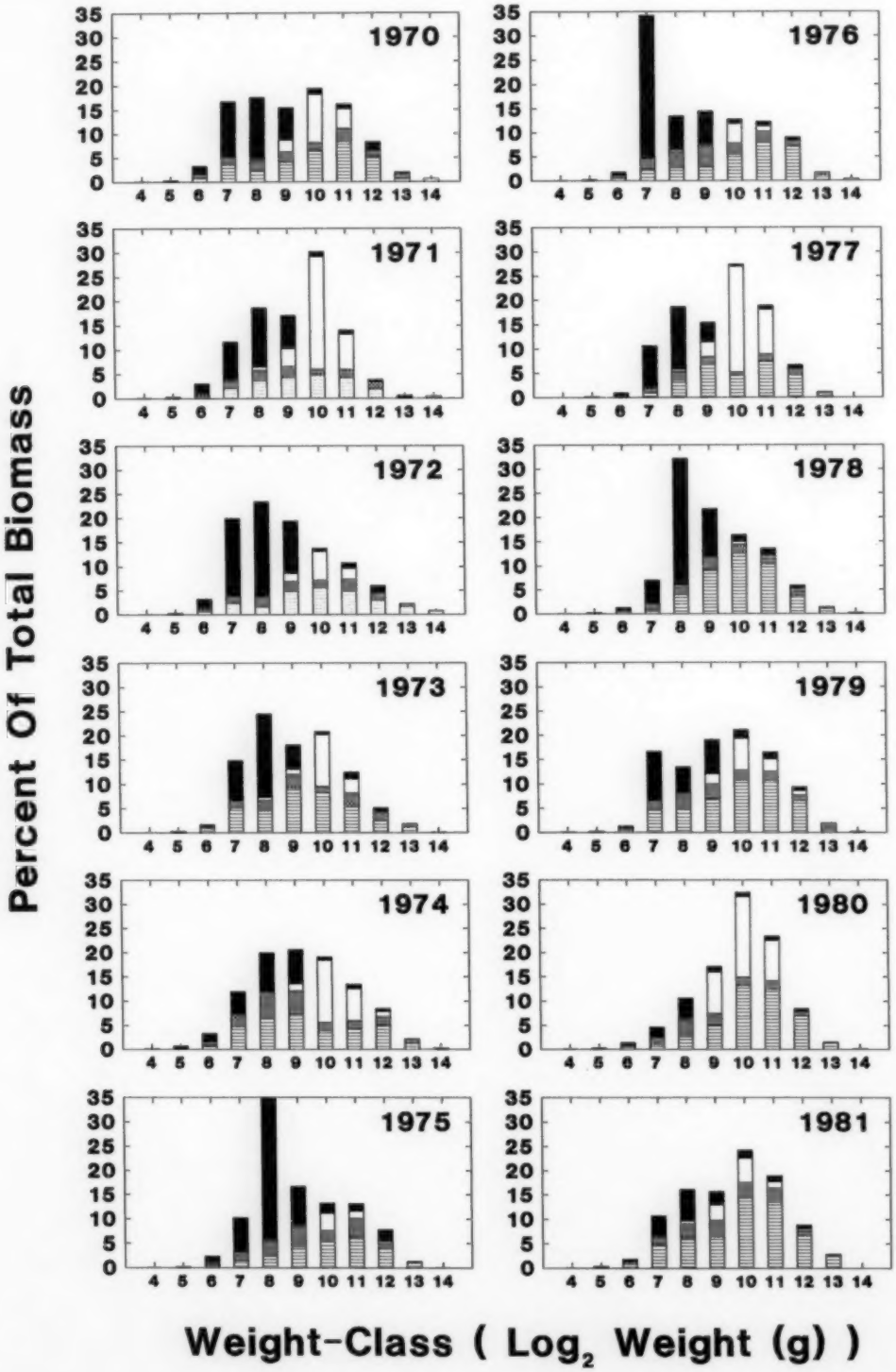

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Fig. 2 (*concluded*).

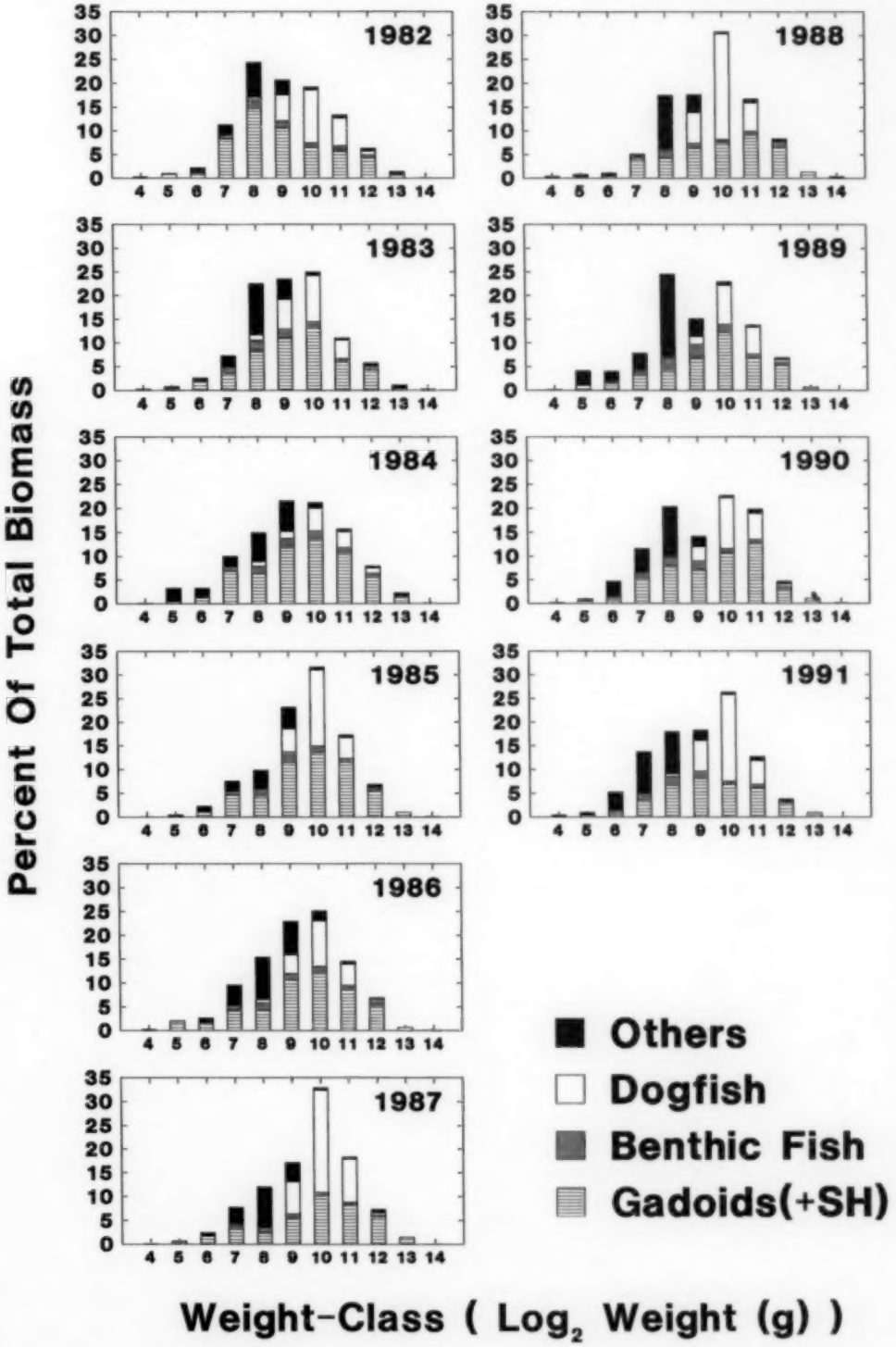

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Table 1. Total biomass (kg·haul^{-1}) of demersal fish species groups on the Scotian Shelf from 1970 to 1991.

Year	Gadoids (–SH)	Silver hake	Dogfish	Flounders	Skates	Others	Total
1970	40.22	8.17	25.49	11.50	8.33	55.82	149.54
1971	28.97	4.79	52.74	10.52	6.23	47.28	150.54
1972	28.53	6.35	13.76	10.11	7.32	70.65	136.74
1973	48.89	11.76	24.57	10.77	10.99	54.73	161.71
1974	33.94	9.78	27.96	17.63	7.18	29.83	126.32
1975	34.48	3.32	7.88	15.41	11.80	75.50	148.39
1976	38.49	4.95	7.79	16.48	8.22	65.39	141.32
1977	57.39	2.67	71.09	10.08	7.39	57.96	206.58
1978	64.00	3.79	3.09	9.63	7.38	70.92	158.81
1979	54.76	10.98	17.90	13.71	7.41	38.46	143.21
1980	66.31	3.85	54.41	13.70	5.71	16.39	160.37
1981	73.14	12.83	16.23	15.69	9.25	26.71	153.85
1982	94.46	34.27	57.86	11.79	6.25	37.68	242.30
1983	90.85	13.87	44.59	11.36	8.08	42.71	211.46
1984	118.41	25.06	28.88	13.26	6.90	53.08	245.59
1985	119.79	19.71	67.85	13.01	7.29	33.28	260.93
1986	85.68	18.94	40.41	9.18	5.68	49.58	209.47
1987	77.05	16.06	91.58	8.07	3.15	40.50	236.41
1988	78.63	13.27	80.93	7.44	5.39	41.59	227.25
1989	61.16	9.69	28.03	10.89	6.31	52.76	168.85
1990	77.52	12.92	35.73	9.08	4.17	40.98	180.41
1991	57.09	10.24	56.76	9.77	3.29	46.86	184.01

Note: One standard haul of a 12 m wide trawl over a distance of 1.75 nautical miles is approximately equivalent to an area of 39 000 m^2. –SH, excluding silver hake.

(250–1000 g). The others group constituted a large proportion of biomass only in weight-classes 4–9 (500–1000 g). In the weight-classes <9 (<500 g), others biomass contribution exceeded that of all the other groups in most years, accounting for as much as 86% of biomass density in weight-class 7 (125–250 g) in 1976.

Combined species size spectra have a characteristic dome shape in all years (Fig. 2). The mode most often occurred in weight-class 10 (1–2 kg), but also appeared sometimes in weight-class 7, 8, or 9, especially in the early 1970s and in 1989 when overall biomass density was in its lower range. Maximum modal biomass was above 35% in 1975 and 1976 but more commonly was about 20–30%. Most of the variation in the spectra was caused by differences in proportional biomass in weight-classes smaller than class 9 (<500 g). This variation in the smaller weight-classes was mostly caused by fluctuations in the proportional biomass contribution of the others group.

Skate total biomass contribution was highest in 1975 at 8%, but more often accounted for only 2–5% of the total fish biomass (Fig. 3). Similarly for flounders, the other benthic fish component, the peak biomass contribution of 14% occurred in 1974, the year of lowest average biomass in the 22-year series. On average, flounders accounted for 5–10% of total biomass. Skate and flounder biomasses closely tracked one another as indicated by significant positive correlations between them (Table 2). Flounder and skate summed as benthic fish, accounted for a maximum of 20% of the total fish biomass in 1974 but only 5% in 1987. Benthic fish most often made up about 10% of the total fish biomass.

Dogfish proportional biomass was highly variable between years and showed no consistent long-term trends (Fig. 3). Dogfish proportional contribution to total biomass was as little as 2% in 1978 but as much as 39% in 1987, the year of second highest overall biomass. Dogfish biomass was significantly negatively correlated with flounders, skates, and others but was not significantly correlated with either gadoids (–SH) or silver hake (Table 2).

Gadoids (–SH) contributed the most of any species group to the total fish biomass on the Scotian Shelf in most years (Fig. 3). Gadoids (–SH) proportional biomass contribution was as little as 19% in 1971, during the period of generally low density, but peaked at 48% in 1984, a year of high overall abundance. Silver hake biomass was always less than 15% of the gadoid fish total in all years. Silver hake and gadoids (–SH) biomass closely tracked each other, as indicated by the positive correlation between their biomasses (Table 2). In the early 1970s, gadoids (–SH) were sometimes less important biomass contributors than dogfish or others, but from about 1980 onward, gadoids (–SH) were the most important contributors to total biomass.

Proportional biomass of others was quite variable between years but showed two phases in its proportional biomass contribution (Fig. 3). Others proportional biomass contribution averaged 39% between 1970 and 1978, when total abundance was low, and 20% between 1979 and 1991, inclusive, when the total was highest. The mean total biomass values in these two phases of the total period were significantly different despite the large interannual variations (t-test, $P < 0.005$).

Analysis of variability in three parameters

Total biomass (ΣB)

Temporal variability, $CV_{\Sigma B}$, was greater among species groups than for their total. Only the ecological grouping benthic fish had lower variability (Table 3). Silver hake and dogfish annual

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Table 2. Pearson correlation coefficients of demersal fish biomass on the Scotian Shelf for 1970–1991.

	Gadoids (–SH)	Silver hake	Dogfish	Flounders	Skates
Silver hake	0.75*				
Dogfish	0.42	0.31			
Flounders	–0.19	–0.11	–0.51*		
Skates	–0.29	–0.24	–0.62*	–0.54*	
Others	–0.39	–0.35	–0.47*	–0.16	0.38

Note: Regressions were calculated from one point for each year, i.e., $n = 22$. *$p < 0.05$. –SH, excluding silver hake.

Table 3. Temporal variability of total biomass density (ΣB, kg·haul^{-1}), modal body weight ($\bar{W}$, $\log_2$ weight (g)), and shape of the logarithmic biomass distribution by size measured as the index of dispersion (I) for demersal fish species-groups on the Scotian Shelf from 1970 to 1991.

	ΣB_{avg}	$CV_{\Sigma B}$	$\bar{W}_{avg}$	$CV_{\bar{W}}$	I_{avg}	CV_I
All species	183	0.22	7.49	0.04	0.32	0.29
Gadoids (–SH)	65	0.40	8.60	0.03	0.28	0.34
Silver hake	12	0.65	6.57	0.05	0.17	0.25
Dogfish	39	0.63	9.59	0.03	0.10	0.77
Flounders	12	0.23	7.20	0.03	0.25	0.11
Skates	7	0.29	7.47	0.06	0.60	0.18
Others	48	0.31	7.17	0.06	0.19	0.10
Gadoids (+SH)	77	0.42	7.61	0.05	0.36	0.28
Benthic fish	19	0.22	7.24	0.03	0.32	0.10

Note: Temporal variability is measured as the coefficient of variation (CV = standard deviation/mean). The subscript "avg" means the arithmetic mean over the 22-year time series: one point for each year. +SH, including silver hake; –SH, excluding silver hake.

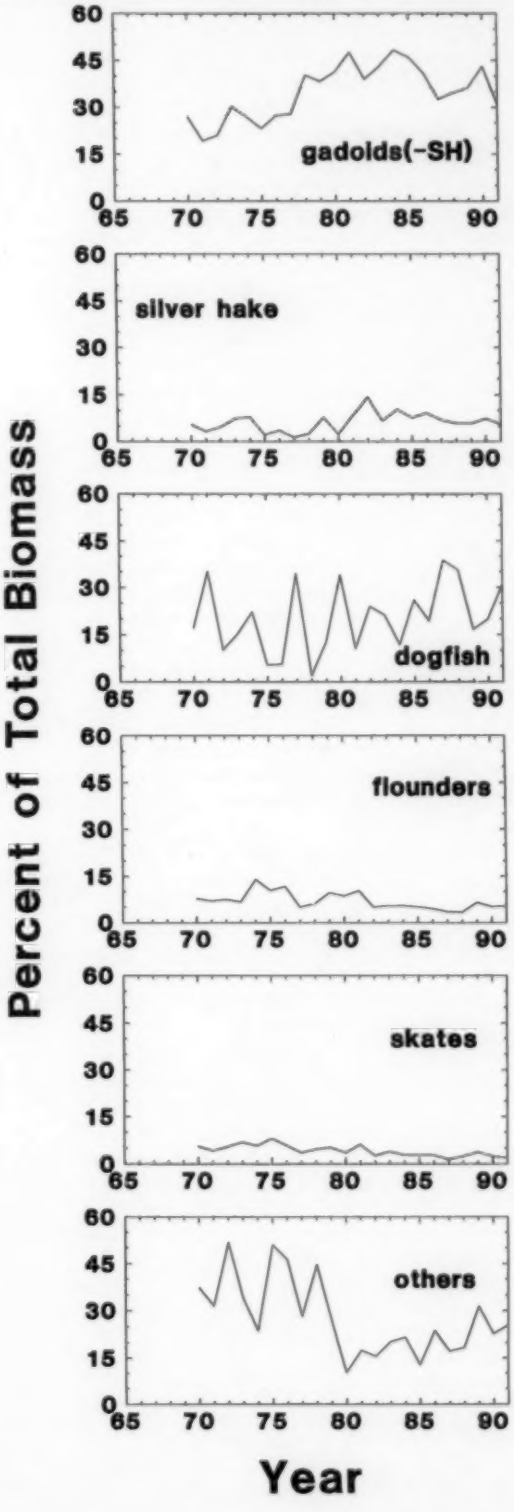
biomasses were equally variable, and were the most variable of all the groups examined. Gadoids (–SH) were the third most variable group examined but still showed only about half the variability of silver hake or dogfish. Including silver hake with the gadoids (–SH) to form an all gadoids (+SH) group increased the CV by only a small amount over the gadoids (–SH) CV even though silver hake variance was relatively high. Flounders and skates were the least variable of any of the individual species groups examined. Grouping flounders and skates into the ecological group benthic fishes decreased the variance even more. The others group, which is highly variable in total numbers, displayed an intermediate level of variability in biomass but below that of the dominant gadoids.

Modal body weight ($\bar{W}$)

The modal body weight of all fish species was 180 g, which was within the range of modes (95–770 g) calculated for groups (Table 3). Dogfish had the largest modal body weight and silver hake the smallest. Flounders, skates, and others all had a similar modal weight. Gadoids (–SH) had a large modal weight closer to that of dogfish.

The silver hake, skate, and others mode was more variable than the gadoids (–SH) mode (Table 3). The dogfish and flounders mode was less variable than the overall mode. When a general gadoid group was composed, its mode (gadoids (+SH)) was more variable than the modes of either of its component groups (silver hake and gadoids (–SH)). Although the silver hake biomass was low relative to that of the gadoids (–SH)

Fig. 3. Proportion of total demersal fish biomass on the Scotian Shelf from 1970 to 1991 partitioned among four species groups.



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(Table 1, Fig. 3), its biomass was highly variable and therefore increased the variability of the gadoids (+SH) mode over that of gadoids (–SH). Temporal variability of the combined benthic fish modal weight was equal to that of flounders but less than that calculated for skates, i.e., flounders were more influential than skates in determining its variability.

Shape of the spectrum measured as index of dispersion (I)

Values of the index of dispersion $I < 1$ indicate that a distribution covers a greater range of values on the body size or x axis than would a normal distribution with the same mean; that is, the distribution appears wide with a low modal biomass value. Thiebaux and Dickie (1992) pointed out that removal of logs from their theoretical expression for the parabolic form of the biomass domes for all species within a trophic position results in the form of a normal distribution. Departures from normality in the overall and individual species data we examined would provide an index of their departures from theoretical expectation.

We determined that I is less than 1 for all the individual species groups examined, as well as for their total over all species collected (Table 3). This suggests that all of the distributions differ in some measure from a normal distribution. The high body weight range over which biomass is spread in dogfish is indicated by a low I_{avg} value of 0.10 (the average is only 1/10 of the variance). However the CV_I for dogfish distribution was also the highest of any species group (0.77). A high CV_I can result either from fluctuations in size range over a constant total biomass, or, what is closer to fact, independent fluctuations in biomass over small parts of the range of weight-classes. While the dogfish exhibited a relatively limited, large size range, this variability in the shape of their biomass size distribution may be related to the sexual dimorphism that must be added to the more general variability, expected of the multiaged year-class strength variations indicated by the fluctuating abundance in Fig. 3.

The aggregated gadoids (+SH) (silver hake and gadoids (–SH)) shows an increase in I_{avg}, compared with either one of its components. This suggests that the effect of adding the silver hake body-weight range to that of gadoids must constitute an overlapping of sizes, hence adding proportionately to their relative biomass. Temporal variability of I for total gadoids (+SH) was intermediate between that of silver hake and other gadoids (–SH).

Of all groups, the skate weight distribution had the largest I value (0.60) compared with all other groups (Table 3); the opposite extreme from the dogfish distribution. Correspondingly, CV_I for the skates was relatively low (0.18), a characteristic that seems also to be reflected in their low, invariable abundance fluctuations. This appears to confirm our inference that I_{avg} is markedly affected by abundance fluctuations in these multiaged populations and is especially marked when sexual dimorphism in body size is present.

The I_{avg} for flounder of 0.25 was in about the midrange of values reported for other groups, and variability of the shape of the flounder distribution was the lowest of all the species groups (Table 3). Aggregate CV_I for benthic fishes, however, was lower than observed for either of its constituents, flounders and skates. That is, the shape of this ecological grouping's size distribution was more stable than either of its component species groups taken separately. The positive correlation observed between skate and flounder abundance reinforces this pattern. The other group also had a low I_{avg} value although one well within the range of the other species groupings and also shows low variability.

In combination, all species combined had an I_{avg} value within the range found in this study. It is, however, close to the value for all gadoids, reflecting the fact that their combined body-size range largely represented the whole. The fact that variability of the shape of the all species size distribution (CV_I) was within the range of CV_I values observed at the level of species group appears to reflect the fact that no one species group exerted an exceptional effect on the total population balance at any time.

Discussion

The hypothesis that total biomass is a system-level phenomenon and therefore is more stable at the combined species level than at the species-group level is supported by the data. The coefficient of variability of total biomass was lower than seven of the eight subsystem level components examined and equal in value to the eighth. The component with equal variability was the benthic fish group, which appeared to act as a stable guild or subsystem of the demersal fish. If there are tight functional constraints (e.g., morphology of feeding apparatus) on a subgroup such as the benthic fish and there is a stable food supply, then it is possible that they will have more stable biomass than a larger suite of species utilising a wider range of energy sources.

Biomass size spectra of combined species on the Scotian Shelf showed greater stability than the individual species-group biomass size spectra. Combined-species size spectra represented the total demersal system biomass within the weight-classes studied. It is reasonable to assume that temporal variability at that scale will be determined by variability of biological energy flow and physical and chemical properties occurring at the same scale (Denman et al. 1989; Steele 1991; Schneider 1994). Biological energy flow and water movement can be traced to weather patterns, nutrient concentrations, and solar energy input integrated over a large area, all of which affect the phytoplankton at the base of the food web (Denman et al. 1989; Kiørboe 1993). Boudreau and Dickie (1992) found strong indications that the level of nutrient supply among areas affected biomass density levels. Demersal fish data from the Scotian Shelf indicated that total biomass (ΣB) as conserved when examined at the total demersal fish community level, thus supporting the view that total biomass is an ecological phenomenon that is conserved at the system scale. Modal body weight ($\bar{W}$), a population-level phenomenon considered to have evolutionary origins, was not more stable at the species-group scale, suggesting that it also owes more to trophic positioning than to the historical variations afforded by evolutionary processes. However, the weight distributions for the multiaged species and communities studied here are considerably more dispersed with body size than would be expected from normal distributions. This may well indicate that multispecies fish populations in their natural environment may represent several overlapping predator–prey energy transmission systems, rather than the dominant single predator–prey body-size ratio envisioned in the original Thiebaux and Dickie (1992, 1993) model and verified by Sprules and Goyke's (1994) fitting of the pelagic system of the Great Lakes.

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Pope et al. (1988) showed that numerical density of demersal fish species groups fluctuated widely between the 1960s and 1980s in the North Sea and on Georges Bank, while at the same time the length–frequency distributions of combined species remained constant. While one needs to exercise caution in comparing length–frequency and weight–biomass distributions, a similar phenomenon is certainly reflected in the demersal fish biomass from the Scotian Shelf. Our results suggest that total biomass within the demersal fishes is bounded at a maximum achievable level, thus maintaining an overall constancy of biomass, but that biomass between species groups can vary within the fish community. Species replacement or compensation is a probable explanation for this pattern, although evidence of its occurrence is difficult to find let alone to deduce possible mechanisms by which it comes about (Daan 1980; Rothschild 1991; Frost et al. 1995).

Pope et al. (1988) presented strong evidence that on Georges Bank, dogfish replaced the gadoids, which were heavily exploited by the commercial fishery. Dogfish may also have replaced some species on the Scotian Shelf, but apparently not gadoids, at least to the same extent as on Georges Bank. Total biomasses contributed by gadoids (–SH) and dogfish on the Scotian Shelf were not significantly related during the period covered by this study. However, the two groups combined appear responsible for the generally higher biomass levels and generally more parabolic-shaped fish domes since the start of the 1980s. Gadoids (–SH) covered a much larger size range than dogfish; therefore, they may also exploit a greater range of prey sizes and a greater number of prey species. The low apparent interaction between gadoids (–SH) and dogfish on the Scotian Shelf may partly result from the high variability in dogfish biomass during the 1970s, when gadoid abundance was especially low.

It is clear from Figs. 1 and 2 that the aggregate pattern of the Scotian Shelf fishery evinces some attribute of coherent system behaviour. Weiss (1969) offered a definition of "system," by proposing that the system criterion is satisfied where the variance of the aggregate whole is very much less than the summed variances of its aggregate parts. That is, following Weiss, we may write:

$$(4) \quad V_s << \sum(V_a + V_b + V_c + \ldots V_n)$$

where V is variance and the subscript s stands for system, and $a, b, \ldots, n$ for its components (species or species aggregates in the present instance). Inspection of Fig. 2 and Table 3 indicates that the Scotian Shelf data meet the Weiss criterion for system behaviour.

In our general data compilation, no obvious taxonomic replacement of demersal fishes occurred on the Scotian Shelf as it did on Georges Bank. As a stronger test for compensation or replacement, we have applied the procedure for determining variance compensation employed by Frost et al. (1995), which is the statistical method for calculating total variance from component variances. The following formula was used with the data in Table 1:

$$(5) \quad \mathrm{Var} \sum_{i=1}^{n} B_i = \sum_{i=1}^{n} \mathrm{Var}\,(B_i) + 2 \cdot \sum_{i=1}^{n} \sum_{j=1}^{i-1} \mathrm{Cov}\,(B_i, B_j)$$

where B_i is the biomass of group i; B_j is biomass of group j; Var is the variance; and Cov is the covariance. Equation 5 is a mathematical idealization. When fitted with data from an ecosystem, departures of the expression from equality become an index of departure from system integration. That is, the ratio of one side of the expression to the other is a standardized measure of species compensation. If the ratio of the left side/right side is appreciably less than unity, then species compensation is occurring, and if near to or greater than unity, then no species compensation is apparent in the data (Frost et al. 1995). Applied to the Scotian Shelf trawl survey data, the test showed that the ratio is very close to unity, confirming that no systematic species group compensation has occurred over the period in question. While this result may be affected by the number of taxonomic groupings considered, since our groupings were chosen for comparability with the Georges Bank fishery where species replacement was apparent, it seems safe to conclude that the level of taxonomic compensation must be less than on Georges Bank.

There are, however, a number of features of the species changes on the Scotian Shelf that have not been clear from previous analyses. Proportional biomass density of skates was low, and therefore, they may not appear important in the demersal fish system of the Scotian Shelf. Nevertheless, for ecological reasons of similar body form and lifestyle, the positive relationship between proportional biomass of skate and flounder may be considered pertinent; these groups combined represent most of the strictly benthic fish biomass on the Scotian Shelf. This benthic fish group may represent an important energy flow pathway from the benthos and at least one component of the demersal fish. That is, benthic invertebrates make up a considerable portion of the diet of flounders on the Scotian Shelf (Martell and McClelland 1994), which represent an energy flow from the benthic system to the demersal fish system. It is probable that size structured interactions occur between the benthic fishes and the benthic invertebrates (Boudreau and Dickie 1992), but little research has been directed at discerning these interactions. What is of interest here is the high stability of these two groups throughout a period when the total biomass changed by a factor of two. It would seem that whatever causes were responsible for the overall biomass change on the Scotian Shelf, it was largely independent of the more strictly benthic component of the demersal fishes.

The two phases in total biomass of the others group (1970–1978 and 1979–1991) corresponded well to two phases in the variability of a biomass size spectrum model parameter, γ, fitted to these data (Duplisea and Kerr 1995). γ is an index of the width of the fish dome and therefore an index of potential food availability. The fitted γ before 1980 was highly variable but after 1980 it was stable and similar to a value derived from the 22-year averaged data. It is of some interest that these phases are roughly separated by the time of implementation of the 200-mile (1 mile = 1.602 km) sovereignty limit of coastal waters in 1977. It brought about large changes in fish management and commercial fishery practices (Angel et al. 1994). Because the two phases are only readily evident in the biomass of the others group, it is difficult to assess the fishery impact on that multispecies-group as a whole. However, it is conceivable that the tighter fisheries regulation after 1977 meant that commercially desirable species of the coastal state, Canada, became more abundant, possibly even at the expense of the others, which formed the large fraction of the lower abundances.

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Gadoids (–SH) show an increase in biomass roughly corresponding to the decrease in others biomass. International fishing fleets on the Scotian Shelf before 1977 directed their considerable fishing efforts at many more species than did the Canadian fleet after 1977 (Angel et al. 1994). Pope et al. (1988) also found that the slope of combined species length–frequency spectra were sensitive to changes in fisheries management. It may therefore appear that the differences between the two periods were more related to conditions associated with exploitation than to other environmental causes. Demersal fishes on Georges Bank show similar patterns of variability in the total and components that seem attributable to the implementation of the 200-mile limit (Rothschild 1991; Mayo et al. 1992).

These analyses applied to systems with collapsed fisheries, such as the Scotian Shelf after 1994 and the Grand Banks after 1993, could provide further insights into the ecological size-based nature of demersal fish systems.

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In situ evaluation of options for chemical treatment of hepatotoxic cyanobacterial blooms

Angeline K.-Y. Lam and Ellie E. Prepas

Abstract: Closed-bottom limnocorrals were placed in a hardwater lake in central Alberta to compare the effect of two alternative approaches to chemical removal of toxic phytoplankton blooms. Reglone A, which lyses phytoplankton cells, and lime–alum, which precipitates intact phytoplankton cells out of the water column were both effective in removing phytoplankton from the water column. Our results were consistent with laboratory studies in that treatment with Reglone A removed phytoplankton (primarily cyanobacteria) blooms with a concomitant increase in dissolved microcystin (exo-MCYST) and phosphorus concentrations in the surrounding water whereas lime–alum treatment did not. Maximum exo-MCYST concentrations in the water phase of the lime–alum treated limnocorrals were 32-fold lower than those recorded in the Reglone-treated limnocorrals. Treatment with lime alone caused a sharp rise in pH (to >10), and the observed increase in exo-MCYST was likely due to pH shock. Exo-MCYST concentration in the Reglone-treated enclosures remained high for the duration of the experiment (>5 days). As microcystin did not partition onto lake sediments in laboratory studies, our limnocorrals results were probably a good indication of microcystin dynamics in lakes. Thus, the use of chemicals such as lime–alum that precipitate out intact phytoplankton cells lessens the potential health risk where microcystins are present.

Résumé : Des enceintes limnologiques à fond fermé ont été placées dans un lac d'eau dure dans le centre de l'Alberta pour comparer l'effet de deux approches différentes à l'élimination chimique des proliférations de phytoplancton toxique. Le Reglone A, qui lyse les cellules de phytoplancton, et la chaux–alun, qui précipite les cellules de phytoplancton intactes hors de la colonne d'eau se sont tous deux révélés des méthodes efficaces pour éliminer le phytoplancton de la colonne d'eau. Nos résultats sont en accord avec des études de laboratoire en ce sens que le traitement au Reglone A a éliminé les proliférations de phytoplancton (principalement de cyanobactéries), mais que cette élimination s'accompagnait d'une augmentation des concentrations de microcystine (exo-MCYST) dissoutes et de phosphore dans les eaux environnantes, tandis que ce n'est pas le cas avec le traitement à la chaux–alun. Les concentrations maximales d'exo-MCYST dans la phase aqueuse des enceintes limnologiques traitées à la chaux–alun étaient 32 fois plus faibles que les concentrations enregistrées dans les enceintes limnologiques traitées au Reglone. Le traitement à la chaux a également entraîné une augmentation abrupte du pH (jusqu'à >10) et l'augmentation observée de la concentration d'exo-MCYST était vraisemblablement attribuable à un choc de pH. La concentration d'exo-MCYST dans les enceintes traitées au Reglone est demeurée élevée pendant toute la durée de l'expérience (>5 jours). Étant donné que la microcystine ne s'est pas incorporée dans les sédiments lacustres au cours des études de laboratoire, nos résultats obtenus avec les enceintes limnologiques étaient probablement une bonne indication de la dynamique de la microcystine dans les lacs. Ainsi, l'utilisation de produits chimiques comme la chaux–alun, qui précipitent les cellules de phytoplancton intactes hors de la colonne d'eau, diminue le risque potentiel pour la santé dans les cas où des microcystines sont présentes.
[Traduit par la Rédaction]

Introduction

Cyanobacteria of the genera *Microcystis*, *Oscillatoria*, and *Anabaena* are known to produce a number of secondary metabolites, some of which cause taste and odour problems in drinking water supplies (Matsumoto and Tsuchiya 1988). Recently, concern has grown in the water treatment industry about potential contamination of drinking water supplies by toxic metabolites called microcystins, a family of at least 50 tumor-promoting hepatotoxins (Carmichael 1994) that induce chronic liver damage (Falconer et al. 1983, 1988).

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A.K.-Y. Lam and E.E. Prepas.[1] Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: eprepas@gpu.srv.ualberta.ca

Microcystin-producing cyanobacteria are found in fresh water and, to a lesser extent, in brackish water. The release of intracellular microcystin (endo-MCYST) from toxic cyanobacteria occurs during cell aging (Berg et al. 1987), and there is growing concern that the chemical treatment of toxic blooms may accelerate this process. Dramatic release (as high as 1800 $\mu g \cdot L^{-1}$) of endo-MCYST has been reported in lake water when cyanobacteria were treated with copper sulfate (Jones and Orr 1994). Dissolved microcystins in the water phase (exo-MCYST) are likely to have a direct impact on human health when they are present in drinking water supply lakes. To understand the potential health risks associated with the chemical treatment of toxic cyanobacterial blooms, knowledge of the persistence of exo-MCYST in lake water and the effects of different chemical treatments on the release of endo-MCYST from cyanobacteria is needed.

The four primary degradation routes for exo-MCYST in lake water are hydrolysis, photolysis, biodegradation or biotransformation, and sorption. Exo-MCLR (Microcystin-LR, the most common microcystin analogue) undergoes only slow

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Table 1. Percent composition of freeze-dried lake sediments collected from Little Beaver Lake on 27 May 1992 based on the Wentworth size class distribution with the SediGraph method.

Sediment	Percentage
Clay (<3.9 μm)	52
Fine to medium silt (3.9–31 μm)	44
Coarse silt (32–62 μm)	3.5
Very fine sand (63–125 μm)	0.5

Note: Particle diameters for the sediment classes are given in parentheses.

degradation in water (0.37% per day at 45°C; Wannemacher 1989). Photochemical decomposition of MCLR was observed only in cyanobacterial pigment solutions (>0.005 $g \cdot L^{-1}$; pigment concentration was based on weight of freeze-dried cyanobacteria per litre) when irradiated with natural sunlight; the photodecomposition rate of MCLR increased with increasing pigment concentration. However, MCLR did not photodecompose when irradiated with fluorescent light (Tsuji et al. 1994). Biotransformation of MCLR to nontoxic product(s) was detected after an initial lag period of 2–10 days (Jones and Orr 1994; Jones et al. 1994; Lam et al. 1995*a*); the biotransformation followed first-order kinetics with a half-life <3.6 days (Kenefick et al. 1993; Lam et al. 1995*a*, 1995*b*). The sorption capability of microcystin could also be an important consideration when determining its persistence in the water column of a lake, but no study has yet explored the ability of MCLR to sorb to lake sediments.

The effects of chemical treatments on microcystin release from cyanobacteria have been studied primarily in laboratory experiments with freshly collected phytoplankton. Lam et al. (1995*b*) categorized chemical treatments on the basis of their effect on MCLR release. Group 1 chemicals disrupt cell functions and induce cell lysis; they include copper sulfate (Kenefick et al. 1993), Reglone A, chlorine, and potassium permanganate (Lam et al. 1995*b*). Group 2 chemicals precipitate phytoplankton and leave cells essentially intact; they include lime ($Ca(OH)_2$; Kenefick et al. 1993; Lam et al. 1995*b*) and alum ($(Al_2SO_4)_3 \cdot 14H_2O$; Lam et al. 1995*b*). Group 2 chemicals were recommended for treating toxic phytoplankton blooms because they induce minimal release of MCLR into the surrounding water (Lam et al. 1995*b*). Increased microcystin concentrations in lake water have been reported after in situ copper sulfate addition to toxic blooms (Jones and Orr 1994); however, the impact of other chemical treatments on toxin release from cyanobacterial blooms in a lake setting has not been studied.

Lakes that serve as drinking water supplies are clearly not suitable for whole-lake studies on the persistence of exo-MCYST after chemical addition, as any toxin release would risk the health of those in contact with the water. At the same time, results generated from laboratory experiments with highly concentrated bloom material or purified toxins may not be applicable to a lake setting. Limnocorrals in lakes represent a compromise between these two approaches as the exo-MCYST released would not enter the main body of the lake. Limnocorrals can be used to examine the applicability of laboratory experiments to a more natural setting, as they allow the inclusion of sunlight, natural bacteria populations, and environmentally relevant concentrations of toxic phytoplankton.

In this two-part study, we (*i*) examined the ability of MCLR to sorb to lake sediments and (*ii*) did two in situ experiments with closed-bottom limnocorrals. We evaluated the suitability of Reglone A (group 1 representative), lime, and lime–alum (group 2 representatives) for the control of hepatotoxic blooms of cyanobacteria and determined the persistence of exo-MCYST in lake water following chemical treatment in limnocorrals.

Materials and methods

Sorption experiment

Little Beaver, Driedmeat, and Coal are hardwater lakes located in the Battle River drainage basin in Alberta, Canada. These lakes have been monitored since 1990 as part of a study on the occurrence and seasonal dynamics of toxic phytoplankton in lakes. These three lakes have similar morphometry and water quality (Mitchell and Prepas 1990) and are microcystin prone (Kotak et al. 1995).

Surface sediments were collected with an Ekman dredge on 26 and 27 May 1992 from all three lakes. The sediments were sterilized at 120°C for two 30-min cycles, freeze-dried, and then analysed for organic content (difference between freeze-dried and ashed sediment weight). The partition coefficient of toxicants is a positive function of sediment organic content (Karickhoff et al. 1979), so sediments from Little Beaver Lake, which had a higher organic content than sediment from Driedmeat or Coal lakes (26, 21, and 12% by weight, respectively) were used in the sorption experiment. Water content (difference between air-dried and freeze-dried sediment weight) and composition (Table 1) of Little Beaver Lake sediments were also determined.

Twenty-seven 60-mL aliquots of autoclaved double distilled water (DDW), each containing 100 μL of 40% formalin were placed in 125-mL Erlenmeyer flasks. Purified MCLR was added to each flask to give an initial concentration of 100 $\mu g\ MCLR \cdot L^{-1}$. Two sediment treatments, 5 and 10 $g \cdot L^{-1}$, represent concentrations at the water–sediment interface where sorption is most likely to occur; control flasks had MCLR but no sediment. Nine replicates were prepared for each treatment. The flasks were capped with foam plugs and shaken continuously in the dark at 24 ± 1°C. Three flasks from each treatment were chosen randomly at 0, 24, and 48 h for MCLR analysis. Sediment samples were centrifuged, and MCLR remaining in the water phase was analyzed by reversed-phase high performance liquid chromatography (HPLC) as outlined by Kenefick et al. (1992) with the following modification: the toxin was eluted from the C-18 cartridge in 1 mL HPLC solvent, and an injection volume of 75 μL was used. A two-way analysis of variance (Wilkinson et al. 1992) was used to evaluate the sorption capability of MCLR.

In situ chemical treatments

A toxic phytoplankton bloom (≥2 $mg \cdot L^{-1}$ phytoplankton biomass at 25 $\mu g\ MCYST \cdot g^{-1}$ biomass; dry weight) was needed to provide a detectable supply of exo-MCYST during these experiments. Experiments 1 and 2 were conducted in Driedmeat Lake on 19 July and 16 August 1993, respectively, in closed-bottomed limnocorrals. Driedmeat Lake was selected as the site for the limnocorral experiments because cyanobacterial bloom density and microcystin concentrations were higher than in either Little Beaver or Coal lakes on those dates. Results of the sorption experiments indicated that contact with lake sediment did not affect the concentration of MCLR in water, so the persistence of microcystin in a closed-bottomed limnocorral should reflect that of a natural lake setting.

The limnocorrals were 1.5 m diameter by 1.5 m high and held 2650 L of water. They were made of clear woven polyethylene fitted with a flotation collar of ethafoam logs (10-cm diameter). To keep each limnocorral vertical in the water column, a heavy metal chain was attached around the bottom edge. Each limnocorral was then secured to a plastic frame which was anchored to a dock. Logs anchored to windward and plastic splash guards (30 cm tall) were used in experiment 2 to prevent waves from entering the limnocorrals.

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Lake water was pumped into the limnocorrals from a depth of approximately 0.5 m and allowed to equilibrate for 2 days before chemical addition. (Extra phytoplankton were collected with a conical 64 μm mesh net and added in equal amounts to limnocorrals in experiment 1.) Experiment 1 was done in duplicate with a control and two chemical treatments. Reglone A (15 $\mu L \cdot L^{-1}$ or 22 $L \cdot ha^{-1}$ or 3 μg diquat$\cdot L^{-1}$) represented chemicals that induce cell lysis, and hydrated lime (100 $mg \cdot L^{-1}$) represented chemicals that precipitate cyanobacterial cells (Lam et al. 1995*b*). Two reference sites (2 m from limnocorral area) were sampled to examine differences between water inside and outside the limnocorrals.

Experiment 2 was done in triplicate and substituted lime–alum for the lime treatment used in experiment 1. Alum was added to maintain a pH between 7 and 10, as the water in the limnocorral was separated from the natural buffering capacity of the bottom sediments. The Reglone-treated limnocorrals were treated at days 0 (3 μg diquat$\cdot L^{-1}$), 2 (1.5 μg diquat$\cdot L^{-1}$), and 3 (3 μg diquat$\cdot L^{-1}$). The lime–alum treatment (Babin et al. 1992) was added to the limnocorrals on day 0 (175 and 175 $mg \cdot L^{-1}$ lime and alum, respectively), and day 4 (100 and 50 $mg \cdot L^{-1}$ lime and alum, respectively). Three limnocorrals with no chemical treatment were used as controls for a total of nine limnocorrals. Three reference sites were also sampled.

Integrated water samples (surface to a depth of 0.75 m) were collected from each limnocorral and reference site with weighted Tygon tubing (i.d. 1.6 cm) fitted with a one-way valve. Water samples for nutrient analysis were kept at 4°C, samples for toxin analysis were filtered through Whatman GF/C filters and stored at –20°C. Exo-MCYST was analysed by protein phosphatase inhibition (PP) bioassay (Holmes 1991; Boland et al. 1993; Lambert et al. 1994) with ^{32}P-radiolabelled phosphatase *a* as the substrate and PP-1c as the enzyme. The PP bioassay was used because it detects total microcystin concentrations; this is more relevant to health risk assessment than MCLR alone as cyanobacteria are known to produce several microcystin analogues (Watanabe et al. 1989; Craig et al. 1993). The results of the PP bioassays are given as micrograms of MCLR equivalent per litre of lake water, with a detection limit (in this study) of 0.05 $\mu g \cdot L^{-1}$.

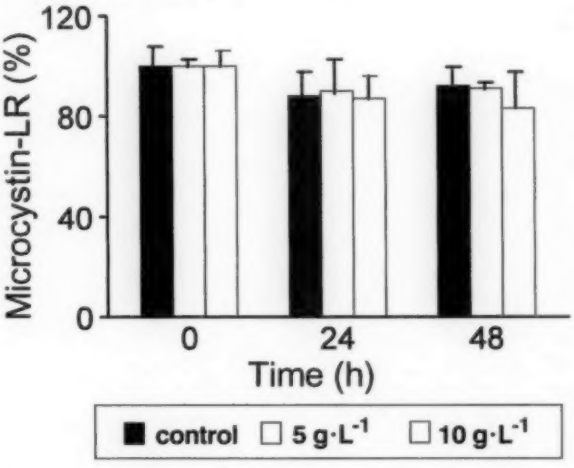
Total phosphorus (TP) and total dissolved phosphorus (TDP; ≤ 0.45 μm) were determined by the persulfate digestion method (Menzel and Corwin 1965) as modified by Prepas and Rigler (1982). Ammonium (NH_4^+; after Solorzano 1969) and nitrate + nitrite (NO_3^-; Stainton et al. 1977) were determined on a Technicon autoanalyzer. Potassium ion (K^+) concentration was measured by atomic absorption (Perkin-Elmer model 3030). Water samples for phytoplankton enumeration were preserved with Lugol's solution and later counted with an inverted microscope by the sedimentation method (Utermöhl 1958). Phytoplankton biomass was then calculated from cell counts and average cell size. Chlorophyll *a* (Chl*a*) was analysed by ethanol extraction (Bergmann and Peters 1980).

Phytoplankton were collected for endo-MCYST analysis with a 64 μm mesh conical net (18 cm diameter by 38 cm length) hauled vertically through the water column from a depth of 0.75 m to the water surface; the number of hauls was recorded to estimate the volume of water sampled. Phytoplankton samples were then freeze-dried, extracted twice with 5% acetic acid as described by Kenefick et al. (1992), and analysed by PP bioassay (Holmes 1991).

Experiments 1 and 2, originally intended to run for 14 days, were ended at 4 and 7 days, respectively, by severe storm activity. Samples were collected on day 0 (immediately before chemical addition) and on days 1, 2, 3, 4, and 7. One replicate of each treatment in experiment 1 was destroyed by storm activity, so the results of this experiment are discussed qualitatively.

Repeated measures on analysis of variance ($P < 0.05$) were used to compare exo-MCYST concentrations in experiment 2 (Wilkinson et al. 1992). Correlation analysis was used to examine the relationships between endo- and exo-MCYST concentrations and between exo-MCYST and other nutrients in lake water (Wilkinson et al. 1992).

Fig. 1. The mean concentration of MCLR in the double-distilled water after exposure to lake sediment is expressed as a percentage of initial MCLR. Two sediment concentrations, 5 and 10 $g \cdot L^{-1}$ were used in this experiment. Error bars are 1 SE.



Results and discussion

Sorption experiment

The water content of surface sediments from Little Beaver Lake was 89%. Freeze-dried sediments had an 26% organic content. Little Beaver Lake sediments were composed of mainly clay and silt (52 and 48%, respectively, based on Wentworth size class distribution; Table 1). When purified MCLR was added to sterilized, freeze-dried lake sediments, no reduction ($P > 0.99$) in dissolved MCLR concentrations in the water phase of both the 5 and 10 $g \cdot L^{-1}$ sorbent concentrations over the 24- and 48-h periods (Fig. 1). This concurs with the findings of Lindholm et al. (1989), who reported that no toxin was detected in the surface sediments of their cyanobacterial-prone study lake. As MCLR did not sorb to sediments, MCYST dynamics in the closed-bottom limnocorrals should be similar to those in a natural lake and that losses of MCYST from the water column are likely due to biotransformation and (or) photolysis.

In situ chemical treatments

At day 0 of experiment 1, phytoplankton biovolumes in the control, lime-treated, and Reglone-treated limnocorrals and at the reference site were similar and exo-MCYST concentrations were below detection in the limnocorrals (Fig. 2). The dominant phytoplankton was the cyanobacterium *Aphanizomenon flos-aquae* (80–90% by volume) while *Microcystis aeruginosa* made up <3% (or 0.5 $mm^3 \cdot L^{-1}$) of the phytoplankton community. Average pH of the lake water at day 0 was 9.2 and did not change much in the control and Reglone-treated limnocorrals, but the pH of the water increased to 10.8 in the lime-treated limnocorrals and remained ≥10 for the following 3 days. Phytoplankton biomass decreased substantially in the Reglone-treated and lime-treated limnocorrals (Fig. 2), while exo-MCYST concentrations increased from below detection (<0.05 $\mu g \cdot L^{-1}$) to maximum concentrations of 0.32 and 0.42 $\mu g \cdot L^{-1}$ in the lime- and Reglone-treated limnocorrals,

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Fig. 2. Exo-microcystin concentrations and phytoplankton volumes in limnocorrals and reference sites over the 4-day period and just prior to chemical addition (day 0) in experiment 1.

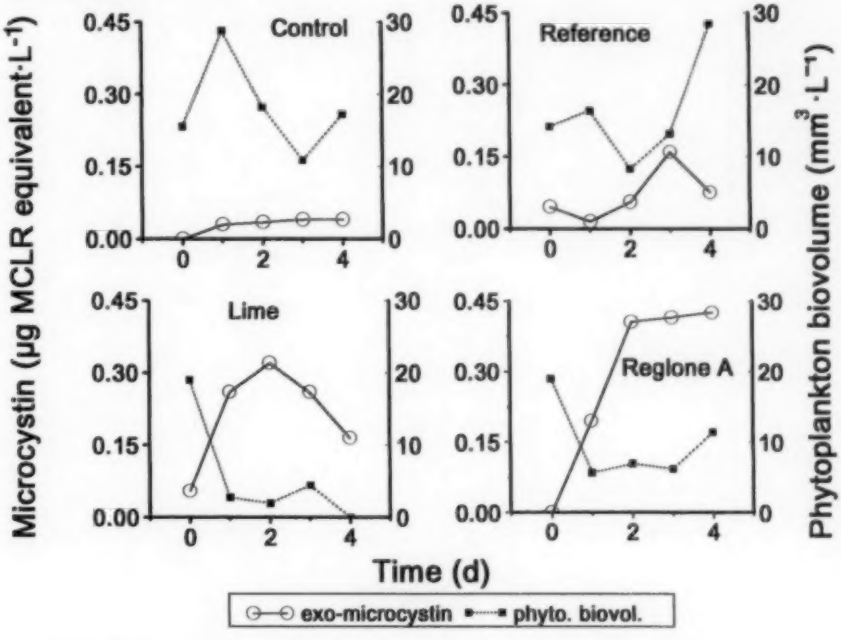

Table 2. Summary of the characteristics of the control, lime–alum, and Reglone-treated limnocorrals and the reference sites in experiment 2 before chemical additions.

	Control	Lime–alum	Reglone A	Reference
Chlorophyll *a* (µg·L^{-1})	65±19	69±15	91±19	33±1.0
Phytoplankton (mm^3·L^{-1})	42±6.1	42±6.4	45±4.0	13±1.7
M. aeruginosa (%)	37	26	22	64
Aph. flos-aquae (%)	62	73	77	32
Endo-MCYST (µg·L^{-1})	0.66±0.24	0.73±0.41	1.09±0.33	1.52±0.20
Exo-MCYST (µg·L^{-1})	0.29±0.06	0.33±0.00	0.32±0.01	0.25±0.06

Note: Values are means ± SE.

respectively. Exo-MCYST concentrations changed minimally both in the control limnocorral and at the reference site over the 4-day experimental period. Phytoplankton biovolumes increased substantially at the reference sites and slightly in the controls during this same period. Overall, the addition of lime (pH >10) or Reglone A removed cyanobacteria but caused a concomitant increase in exo-MCYST concentrations. Previous studies of lime treatment recommended maintaining a water pH <10 to avoid pH shock to aquatic life (Murphy and Prepas 1990); our results indicate that pH shock to toxic cyanobacteria can cause the release of the toxin and should be avoided when lime is used to control cyanobacterial blooms. (In natural lakes, the buffer capacity of the sediments reduces the severity of the pH increase after lime addition.) The lime–alum treatment used in experiment 2 solved this problem: post-treatment pH in limnocorrals and reference sites were similar (9.6 and 9.3, respectively).

Pretreatment characteristics of all the limnocorrals and reference sites used in experiment 2 are summarized in Table 2. Exo-MCYST concentrations were similar in the limnocorrals and reference sites, despite a two- to three-fold difference in Chl*a* and biomass. This suggested that the integrity of *Microcystis* cells was similar among all limnocorrals and at the reference sites at the beginning of experiment 2.

Over the course of the 7-day experiment, Chl*a* concentrations were correlated with phytoplankton volumes in the control, lime–alum, and Reglone-treated limnocorrals (r = 0.86, 0.97, and 0.84; n = 18, 17, and 17, respectively; $P < 0.001$) and at the reference sites ($r = 0.71$; $n = 18$; $P < 0.001$). Chlorophyll *a* concentration was therefore a suitable indicator of phytoplankton biomass in these experiments. Post-treatment Chl*a* concentrations in both the lime–alum and Reglone-treated limnocorrals dropped to <5 µg·L^{-1} (a 20-fold decrease). Phytoplankton biovolumes also decreased dramatically in both lime–alum and Reglone-treated limnocorrals (Fig. 3).

The decrease in Chl*a* concentrations in both lime- and Reglone-treated limnocorrals suggests that both chemicals remove phytoplankton effectively. As lime–alum treatment sediments *Microcystis* cells, post-treatment *Microcystis* volume was lower by an order of magnitude in the lime–alum treated limnocorrals than in the Reglone-treated limnocorrals for the duration of the experiment. When examined with an inverted

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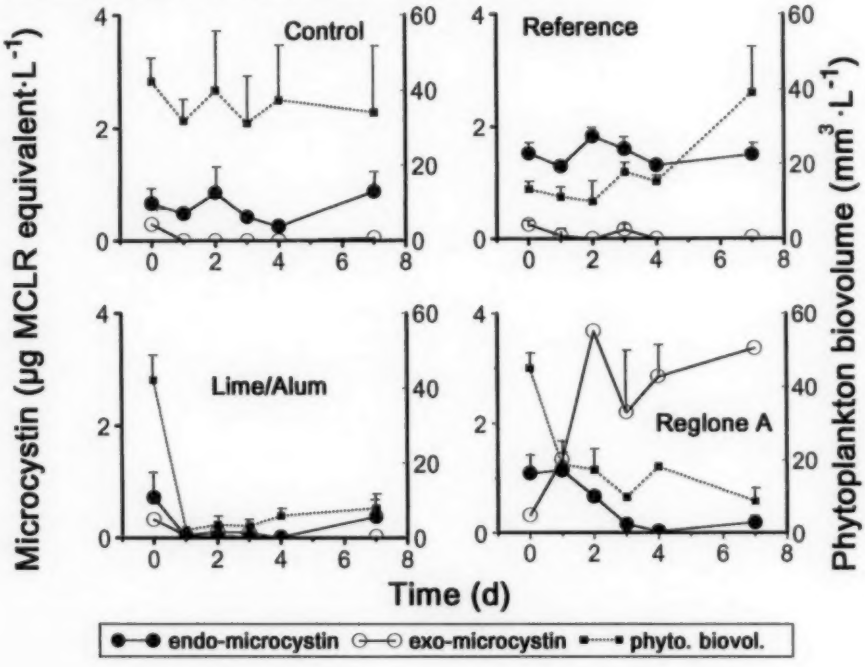
Fig. 3. Average endo- and exo-microcystin concentrations and phytoplankton volumes in all limnocorrals and reference site over the 7-day period and just prior to chemical addition (day 0) in experiment 2. Error bars are 1 SE.



microscope, the *Microcystis* cells in the Reglone-treated limnocorrals did not have a clear cell structure and were probably lysed cells that were resuspended into the water column.

Trends in both endo- and exo-MCYST concentrations, which fluctuated over the 7-day study period, were similar between the control limnocorrals and the reference sites (Fig. 3). In the control limnocorrals, post-treatment concentrations of endo-MCYST remained consistantly higher than exo-MCYST; microcystin appeared to be retained inside cells in both the control limnocorrals and reference sites. In the lime–alum treated limnocorrals, post-treatment endo-MCYST concentrations decreased to below detection on day 1, remained the same from days 2–4, and increased to slightly above detection by day 7 (Fig. 3). Pre-treatment exo-MCYST concentrations in the lime–alum treated limnocorrals decreased to the detection limit on day 1, climbed slightly between days 2 and 3 and then fell below detection again. In the Reglone-treated limnocorrals, average endo-MCYST concentrations declined gradually to below detection on day 4 and increased slightly by day 7. Meanwhile, exo-MCYST concentrations in the Reglone-treated limnocorrals increased 11-fold to a maximum of 3.7 $\mu g \cdot L^{-1}$ on day 2 and remained at high concentrations for the next 5 days. The lytic action of Reglone A removed toxic blooms of *M. aeruginosa* with a concomitant 10-fold increase in exo-MCYST concentrations in the surrounding water compared with pretreatment concentrations (Fig. 3). Exo-MCYST concentrations in the Reglone-treated limnocorrals were on average 29-fold greater than those in the lime–alum treated limnocorrals, whereas the highest detectable exo-MCYST concentration in the lime–alum treated limnocorrals was similar to that at the reference sites. Similar results were obtained in recent laboratory experiments (Lam et al. 1995*b*).

Maximum exo-MCYST concentrations in the Reglone-treated limnocorrals were approximately 330% higher than the initial extractable endo-MCYST. This phenomenon was also observed in laboratory experiments (Lam et al. 1995*b*) and may be a result of a greater toxin release when cells are exposed to Reglone A than that achieved by the extraction with 5% acetic acid used in the PP analysis. (When Reglone A alone was added to PP-1c, no inhibition effect was detected.) This result suggested that many of the hydrophobic microcystins were not extracted in the analysis and total endo-MCYST could likely be underestimated.

The exo-MCYST released in the Reglone-treated limnocorrals remained high from days 2 to 7 and did not appear to degrade. This agrees with the results of a study in Lake Centenary, Australia, where exo-MCYST persisted at high concentrations for 9 days before biotransformation began (Jones and Orr 1994). A similar initial lag phase for biotransformation (7–10 days) was also observed in laboratory experiments when purified MCLR was incubated with microbial from sewage effluent (Lam et al. 1995*a*) or lake water (Jones et al. 1994). Photodegradation of exo-MCYST, a process dependent on the concentration of water-extractable cyanobacterial pigments (Tsuji et al. 1994), was not expected to be an important process in our limnocorrals nor in the lakes as the concentrations of pigments needed for significant photodegradation are far greater than those commonly found in even hypereutrophic lakes. As sorption to lake sediments was insignificant in our experiments, biotransformation remains the most likely means of removing exo-MCYST from the water column. The long

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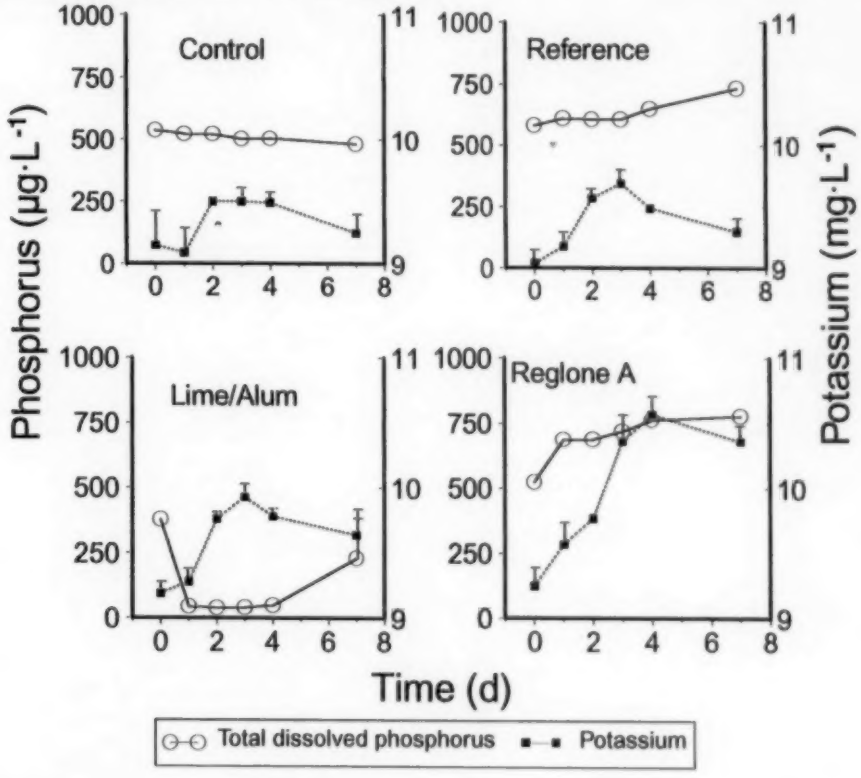
Fig. 4. Average total dissolved phosphorus and potassium concentrations in all limnocorrals and reference site over the 7-day period and just prior to chemical addition (day 0) in experiment 2. Error bars are 1 SE.



lag times may present problems to those seeking to use biotransformation as a means of removing exo-MCYST in water treatment.

Lime–alum application removed phytoplankton with a concomitant decrease in TP and TDP concentrations (Fig. 4), whereas Reglone A did not remove TDP from the water column. (For clarity, TP concentrations are not shown, trends in TP were nearly identical to those of TDP.) At the reference sites, increases in TDP and TP concentrations coincided with increased phytoplankton volumes (Figs. 3 and 4), while average TP and TDP concentrations did not change appreciably (≤5%) in the control limnocorrals over the 7-day period. In the lime–alum treated limnocorrals, both TDP and TP concentrations decreased sixfold on day 1 to 45 and 114 $\mu g \cdot L^{-1}$, respectively. In contrast, TP and TDP remained relatively stable in the Reglone-treated limnocorrals, even though phytoplankton biomass decreased dramatically after treatment.

The lack of change in P concentrations observed in the Reglone-treated limnocorrals was probably the result of cell lysis. In addition to exo-MCYST, nutrients such as P, N, and K are released into the water phase as phytoplankton cells decay; the lytic action of Reglone A likely increases this release. A high degree of correlation ($P < 0.005$) between nutrient parameters such as TDP, TP, NH_4^+, and K^+, and exo-MCYST concentrations in the Reglone-treated limnocorrals supports this idea. The similarities in the patterns of exo-MCYST (Fig. 3) and K^+ (Fig. 4) concentrations are striking. None of these parameters were correlated with exo-MCYST concentrations at the reference sites, nor in the control and lime–alum treated limnocorrals. No clear pattern was observed in the highly variable dissolved inorganic nitrogen (DIN) concentrations in either the limnocorrals or at the reference sites over the 7-day experiment.

The field experiments support the concept that chemical treatments that precipitate or sediment cyanobacteria cells (such as lime–alum) are a better choice than chemicals that cause cell destruction (such as Reglone A and chlorine) for the control of microcystin-containing phytoplankton blooms (Lam et al. 1995*b*). The addition of lime alone resulted in a pH >10 in the limnocorral; the concomitant increase of exo-MCYST was likely due to pH shock. In the hardwater lakes of this region, a similar dosage of lime would not cause the extreme increase in pH observed in the limnocorrals because of the buffering activity of lake sediments (E.E. Prepas and S. Reedyk, unpublished data). Lime–alum removes MCYST-containing phytoplankton effectively without causing an increase in exo-MCYST concentrations in the surrounding water and also appears to remove TP and TDP from the water column. Reglone A treatment removed phytoplankton effectively but resulted in increased TDP, TP, and exo-MCYST concentrations in the surrounding water. As P is the nutrient regulating phytoplankton growth in most lake water (Schindler 1974), lime–alum treatment may also have more of a long-term effect than Reglone A on phytoplankton biomass. Lime–alum treatments

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were more effective than Reglone A in reducing phytoplankton biomass in the poorly buffered stormwater retention lakes in the City of Edmonton (Babin et al. 1992).

In addition, laboratory experiments indicate that lake sediments did not sorb microcystin from lake water, thus the persistence of exo-MCYST concentrations after Reglone A addition is likely to increase chronic health risks in drinking water. A suitable withholding period (»7 days), together with exo-MCYST monitoring is required after Reglone A addition, or after the addition of any other chemical that lyses cells, before the water can be used as a drinking water supply or for contact recreation.

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Patterns in fish assemblages from coastal wetland and beach habitats in Green Bay, Lake Michigan: a multivariate analysis of abiotic and biotic forcing factors

John C. Brazner and Edward W. Beals

Abstract: From May to September in 1990 and 1991, 24 coastal wetland and beach sites in Green Bay, Lake Michigan, were sampled to investigate abiotic and biotic factors influencing fish assemblages; half the sites were modified by human developments, and half were relatively undeveloped. The greatest assemblage differences were observed among regions, but there also were strong differences among assemblages from different habitats. Degree of development had less of an effect on site differences, although assemblages at undeveloped wetlands were unique, and those from developed and undeveloped sites in the upper bay were relatively distinct. The most influential abiotic factors were turbidity, reflecting the trophic gradient in the bay, and a suite of variables associated with macrophyte coverage and diversity, which were critical components of nursery habitats for the primarily immature fishes we captured. The volatile and unpredictable nature of shoreline habitats in the Great Lakes apparently precluded competition and predation from having a strong organizing role. This study demonstrates that undeveloped wetlands are a valuable and intensely utilized fish habitat, particularly as nursery areas, that should receive special consideration in ecosystem management plans for the Great Lakes.

Résumé : De mai à septembre, en 1990 et 1991, 24 sites riverains constitués de terres marécageuses et de plages ont été échantillonnés dans la baie Green, lac Michigan, pour étudier les facteurs abiotiques et biotiques influant sur les assemblages de poissons; la moitié des sites ont été modifiés par l'activité humaine et la moitié étaient relativement peu développés. Les plus grandes différences d'assemblage ont été observées entre les régions, mais il y avait également des différences prononcées entre les assemblages des différents habitats. Le degré de développement avait un effet moins grand sur les différences entre les sites, bien que les assemblages dans les terres marécageuses non développées aient été uniques et que les assemblages caractéristiques des sites développés et non développés dans la partie supérieure de la Baie aient été relativement distincts. Les facteurs abiotiques ayant l'effet le plus grand étaient la turbidité, qui reflète le gradient trophique dans la baie, et une série de variables liées à la superficie couverte par les macrophytes et à leur diversité, qui étaient des constituants déterminants des habitats d'alevinage pour les poissons principalement immatures que nous avons capturés. La nature volatile et imprévisible des habitats riverains dans les Grands Lacs a apparemment empêché la compétition et la prédation d'exercer un rôle important au niveau de l'organisation. Cette étude démontre que les terres humides non développées constituent un habitat précieux et intensément utilisé par le poisson, particulièrement comme aire d'alevinage, qui mérite une attention spéciale dans les plans de gestion de l'écosystème des Grands Lacs.
[Traduit par la Rédaction]

Introduction

Comparative studies of north-temperate lakes indicate that fish assemblages are structured by a variety of abiotic and biotic factors. Two principal abiotic factors implicated in structuring fish assemblages are low pH (Rahel 1986) and low dissolved oxygen in winter (Tonn and Magnuson 1982; Magnuson et al. 1989). Predation has been identified as the principal biotic factor involved in structuring fish communities, although competition for food also appears to play a role (Keast 1978; Tonn 1985). Typically, small or soft-rayed forage species are reduced or absent from lakes where large piscivores are abundant (Robinson and Tonn 1989; He and Wright 1992). Other factors, such as lake area (Johnson et al. 1977; Matuszek and Beggs 1988), habitat heterogeneity (Eadie and Keast 1984; Benson and Magnuson 1992), productivity (Gascon and Leggett 1977; Kelso 1988), and biogeography (Eadie et al. 1986; Jackson and Harvey 1989) may also influence structural patterns. Distinct patterns in fish communities have even been shown to correspond to broad patterns in topography, land use, vegetation, and soils within a watershed (Whittier et al. 1988). Although fish assemblage patterns in relation to biotic and abiotic factors within lakes have been documented (e.g., Werner et al. 1977; Keast and Harker 1977), most conclusions have been derived from comparisons among lakes.

Numerous studies in streams have related fish community structure to human-induced stresses (Reeves et al.1993; Weaver and Garman 1994; Wichert 1995), but relatively few

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J.C. Brazner.[1] U.S. Environmental Protection Agency, National Health and Environmental Effects Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804, U.S.A.
E.W. Beals. Unity College, Unity College, ME 04988, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail epajb@du4500.dul.epa.gov

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have been done in lakes. However, the effects of eutrophication (Schindler et al. 1971; Persson et al. 1991), heavy metals (Somers and Harvey 1984), acid deposition (Schindler et al.1985; Tremblay and Richard 1993), and elevated turbidity (Hayes et al. 1992) on lake fish communities have been examined. With the exception of this study and a few others from around the Great Lakes basin (for review see Jude and Pappas 1992; Brazner 1994), lake-edge wetland and littoral fish assemblages have largely been ignored.

Because the ecology of coastal wetland fishes is not well understood in the Great Lakes, and because degradation of coastal wetlands due to shoreline development and other stressors continues to be a problem (Bosley 1978; Whillans 1982; Krieger et al. 1992), further study of fish assemblages from these habitats seemed warranted. Indications are that a high percentage (>75%) of Great lakes fishes depend upon coastal wetlands for some part of their life cycle (Stephenson 1990; Whillans 1992). Using a univariate, demographic approach, Brazner (1997) found that there were differences in the composition and abundance of fish assemblages related to regions, habitats, and human development intensity in Green Bay coastal habitats. However, the specific factors driving these differences were not identified. Here, our goals were to identify abiotic and biotic factors producing differences in assemblage patterns, using a community-based approach to characterize differences among types of sites and their species structure.

We expected variables associated with trophic status to have an important influence on fish assemblage patterns because a strong trophic gradient is a predominant feature in Green Bay (Sager and Richman 1991), and a similar production gradient had a key role in structuring littoral zone fish communities in Lake Memphremagog (Nakashima et al. 1977; Gascon and Leggett 1977). We also expected that variables associated with site-specific differences in habitat complexity would lead to differences in fish assemblages based on observations made in littoral zones of other freshwater (Guillory et al. 1979, Benson and Magnuson 1992) and coastal marine environments (Heck et al. 1989, Lenanton and Caputi 1989). Although we were unsure what the relative influence of human development might be in this study, we expected the effects of development to be readily apparent because of the high degree of shoreline modification in various parts of Green Bay (Bosley 1978; Harris et al. 1988). Differences in the physical stability of wetland and beach habitats led us to believe that biotic interactions would be more important at wetland sites. High environmental instability, such as the high degree of exposure to wind and waves at beach sites, was expected to decrease the importance of interactions like competion and predation (Menge and Sutherland 1987; Winemiller and Rose 1992) and increase the influence of abiotic factors (Wiens 1984; Jennings and Philipp 1994).

Methods

The study sites, experimental design, and fish sampling methods were described in detail elsewhere (Brazner 1997). Fish assemblages from 24 sites (12 wetland, 12 beach), primarily along the western shoreline of Green Bay, Lake Michigan (Fig. 1), were sampled five times from May to September during 1990 and 1991 with small-meshed (≤5 mm) fyke nets, seines, and minnow traps. Sites spanned the 193 km length of the bay, from the city of Green Bay in the south to the northern end of Little Bay de Noc in the north. Eight sites were selected in each of three geographic regions (lower, middle, and upper bay) to encompass the trophic gradient that is present (Sager and Richman 1991). Half of the sites were among the least developed within each region; the other half were modified by human developments (e.g., residential and (or) industrial development). Sites were 100-m wide, bounded by the shoreline on the inshore edge, and the 1-m depth contour offshore. Coastal wetlands were defined as contiguous, lacustrine habitats less than 2 m deep that had free exchange with the bay and emergent, floating, and (or) submergent vegetation. Beaches were more open, unprotected sites defined by the predominance of fine-textured sand substrates; most had little or no macrophyte cover.

Environmental measurements

Physicochemical variables

Environmental variables expected to influence the littoral fish assemblages in Green Bay were measured concurrently with fish sampling. Water temperature, conductivity, dissolved oxygen and pH were measured at mid-depth along the 1-m depth contour with a Hydrolab 4041 multiprobe sensing unit (Hydrolab Corp., Austin, Tex.) calibrated weekly. Wind speed and direction were determined with a hand held anemometer (Wind Wizard, Davis Instruments, Hayward, Calif.) and wave height was recorded as the mean of the three largest waves measured at the 0.5-m depth contour during a 2-min observation period. Turbidity was estimated in the field from a surface water sample at the 0.5-m depth contour using a model TTM turbidity test kit (LaMotte Chemical, Chestertown, Md.) as Jackson Turbidity Units (JTU). Bottom slope was estimated by averaging the distance from shore to the 1-m depth contour at the two ends and center of each 100-m site transect. Sites were assigned geographic positions (latitude and longitude; Brazner 1997) using a portable Global Positioning System unit (Trimble Transpak, Sunnyvale, Calif.) with a reported accuracy of 33 m. Because of the primarily north–south orientation of the shoreline (i.e., limited longitudinal variability) only latitude was included in statistical analyses. Length of continuous habitat along the shoreline surrounding each site was estimated to the nearest 100 m using a mechanical range finder (Rangematic 1200, Ranging Inc., Rochester, N.Y.).

Substrate variables

Predominant substrate types (comprising ≥25% of total sample) were subjectively determined for each site from three, randomly selected, 1.0-L grab samples taken during August–September in 1990 and 1991. Samples were scored on a seven-point scale (1, sand; 2, sand with muck or silt; 3, sand with cobble or gravel; 4, muck with woody debris; 5, gravel with cobble; 6, sand with silt and muck; 7, sand with muck and cobble or wood, or silt with gravel and cobble or wood) to provide a scale of increasing substrate diversity similar to those calculated by Tonn and Magnuson (1982) and Benson and Magnuson (1992). Substrate samples were frozen and analyzed later for organic carbon using a combustion method (Dean 1974). Sediment diversity and organic carbon estimates are means of values from the 2 years of study. Sediment diversity was rounded to the nearest integer.

Macrophyte community variables

The number of macrophyte species and the percentage of area covered by macrophytes were estimated. Any species observed during a 15-min wading survey between the 1-m depth contour and the daily seiche line was counted. Percentage cover (recorded to the nearest 10%) was a subjective estimate of total areal coverage by all macrophyte species in the designated study areas. A diversity index of macrophyte growth forms (Fassett 1930) similar to the ones described by Tonn and Magnuson (1982) and Benson and Magnuson (1992) was calculated. Macrophyte growth form diversity ranged from 1 to 7 (1, no macrophytes; 2, flexuous forms only; 3, rosette forms only; 4, emergent and flexuous forms; 5, emergent, flexuous, and rosette forms; 6, emergent, floating and flexuous forms; 7, emergent, floating,

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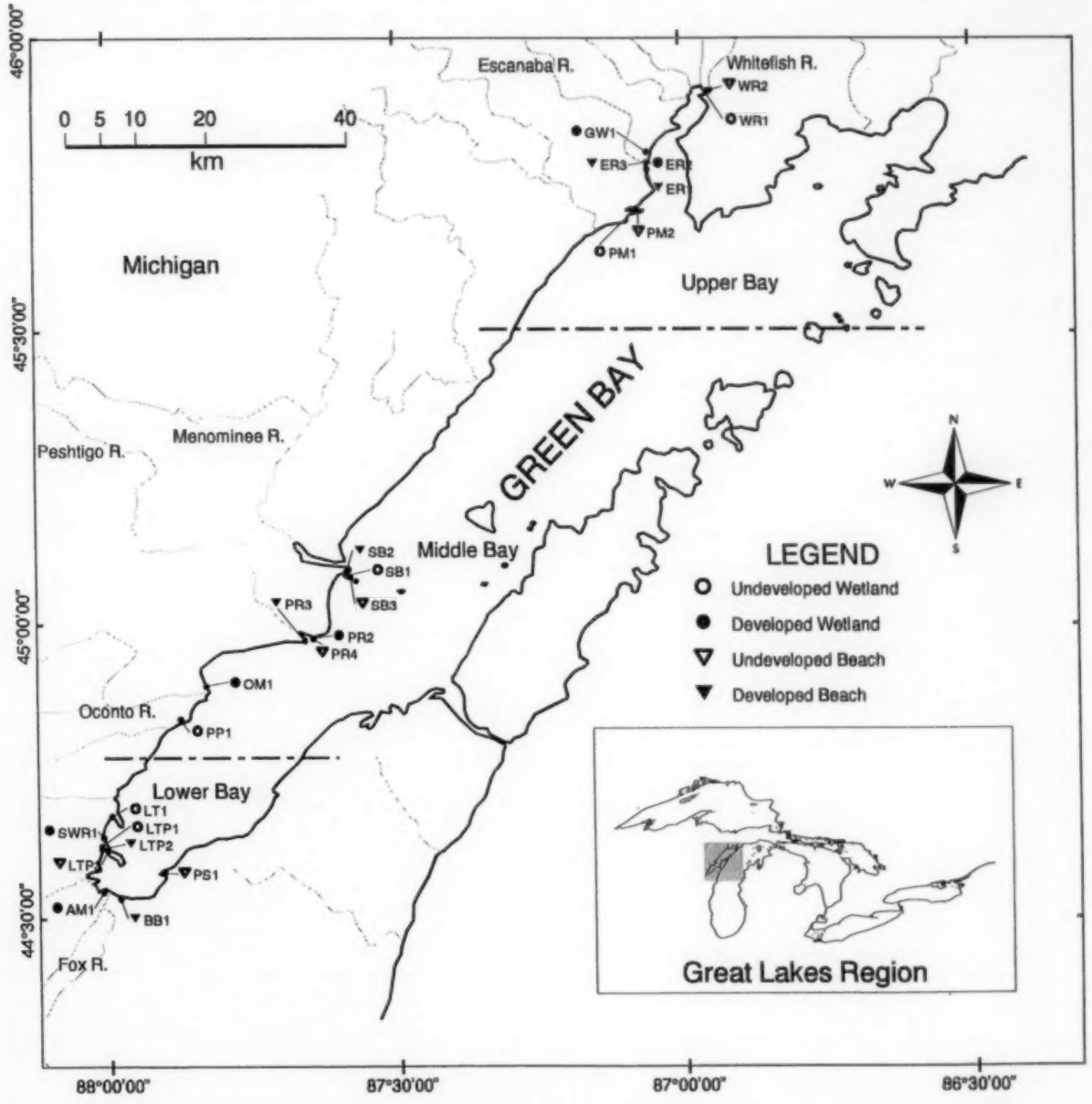
Fig. 1. Map of Green Bay showing its relative position in the Great Lakes region and Lake Michigan, as well as locations of 24 sites sampled during the study (undeveloped wetland sites, ○; developed wetlands, ●; undeveloped beaches, ▽; developed beaches, ▼; more site details are given in the Appendix).



flexuous, and rosette forms); assigning the highest scores to sites with the greatest number of growth forms.

Overall macrophyte diversity was calculated from percentage cover, the number of species and growth form diversity:

$$MD = C(S/MS)(F/7)$$

where MD is macrophyte diversity, C is percent cover, S is number of species, MS is maximum number of species found at any site, and F is growth form diversity. This index of macrophyte diversity potentially ranged from 0 to 1; sites with no macrophytes received a score of 0 and sites with 100% cover, the maximum number of species observed and a growth form diversity of 7 receiving a score of 1. The highest macrophyte diversity actually calculated for any site was 0.74.

Human development variables

Three measures were utilized to characterize human development adjacent to a site. These were (*i*) distance to the nearest city (Green Bay, Oconto, Peshtigo, and Marinette Wis., and Menominee, Escanaba, and Gladstone, Mich.); (*ii*) distance to the nearest federal or state highway measured from 1 : 30 000 scale NOAA navigation maps for

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the Great Lakes printed in 1986, and (*iii*) a relative index of human development within 1.0 km of the center each site. The index ranged from 1 to 4 (1, little or no human development; 4, extreme development) and was estimated from the area and intensity of physical alteration (e.g., landfilling, road building, home and industrial development, cottage use).

Data analysis and summary

Environmental distinctness

To evaluate the differences in environmental conditions at different types of sites, values for each variable averaged over all sampling dates were analyzed with three-way ANOVA using region (lower, middle, upper), habitat type (wetland, beach) and development status (developed, undeveloped) as main factors and a habitat-by-development status interaction term. Fisher's least squared difference (LSD) was used for multiple mean comparisons. All environmental variables were $\log(n + 1)$ transformed to increase variance homogeneity except for latitude, pH, and those based on an index or percent scale.

Fish assemblage structure

We used classification and ordination methods to examine patterns in littoral fish assemblages. Because of their complementary nature, classification and ordination together provide a powerful tool for identifying pattern in the structure of community data (Gauch 1982; Pielou 1984; Ludwig and Reynolds 1988). Several different classification and ordination methods, distance measures, and transformations were tested using data from 1990. These preliminary analyses examined sites in species space, and species in site space matrices to determine which of these analyses would be most suitable. Those techniques that produced the most ecologically meaningful and interpretable patterns were selected for the full data set.

Ward's method was selected as a clustering technique (Lance and Williams 1967; Pielou 1984). The relative euclidean distance measure used with this technique is Wishart's (1969) objective function, which measures information loss as agglomeration proceeds (McCune 1991). A modified version of the Bray–Curtis ordination technique (Bray and Curtis 1957) that incorporates a variance-regression approach for endpoint selection (Beals 1984) provided clearer site separation and greater ecological interpretability than detrended correspondence analysis or nonmetric multidimensional scaling. Sørensen's (1948) coefficient as a distance measure and a $\log(n + 1)$ transformation of abundances provided the best results with this technique. By eliminating species that were rarely captured (fewer than five individuals captured or two or fewer sites of capture) the analytical problems associated with many zeros in the data matrix were reduced (Legendre and Legendre 1983; Tonn et al. 1990). The influence of rare species can obscure analysis of the data set as a whole (Gauch 1982), and rare species are usually the least adequately sampled. Although finding an appropriate reduction in the species data matrix can be problematic and bias results (Rahel et al. 1984; Yant et al. 1984), a reduced matrix (36 species) that omitted rare species was selected because it produced a more ecologically meaningful and workable representation of the data than when all 54 species were included.

Three forms of the data matrix were used for the cluster and ordination analyses to explore patterns in the data set and generate hypotheses about the patterns. A matrix of sites in species space was used to examine similarities and discontinuities among groups of sites based on fish species composition and abundance. To help interpret patterns in the ordinations, product–moment correlations were calculated between positions of sites along each ordination axis and species abundances and environmental characteristics at those sites. Strength of the groupings were estimated with multiple discriminant analysis (Wilkinson 1990). Abundances of the eight fishes most closely correlated with the first three ordination axes were inputs to a discriminant analyses; the number of misclassifications in the site-classification matrices estimated the strength of relationships hypothesized from the ordinations. We limited the number of input variables to eight because the number of samples to be grouped with discriminant analyses should be three to four times greater than the number of predictor variables to assure stability of the discriminant function loadings (Williams and Titus 1988) and that canonical correlations reflect actual underlying relationships (Smith 1981). A matrix of species abundances in site space (the transpose of the first matrix) was used in a cluster analysis to identify which species were associated with particular types of sites.

A third form of the data matrix was used to ordinate species directly in environmental space and determine which abiotic factors were most important to the distribution of individual species. The original site by species matrix was transposed ($\mathbf{A}'$) and postmultiplied by the environmental matrix ($\mathbf{E}$) to produce a matrix ($\mathbf{A}'\mathbf{E}$) of species scores for each environmental variable (McCune 1991). The $\mathbf{A}'$ and $\mathbf{E}$ matrices were first standardized by the mean and standard deviate for each variable. This third matrix provides a direct and efficient way to investigate relationships between species and environment, but like all ordination comparisons with environmental variables, it is constrained by the presumption that the important environmental variables have been measured (Beals 1984; McCune 1991).

Species scores for each environmental variable were overlayed onto the ordination plot to identify which environmental variables contributed most. Ordination points for each species were sized in proportion to their standardized score for each environmental variable (McCune 1991). Contour lines based on these proportionally sized points and judged to correspond with major breaks in the species scores were drawn to provide three or four groups of species. Overlays were plotted for the six environmental variables that were highly correlated with ordination axes and provided clear separation between contours.

Species distributions

The utility of different abiotic factors as predictors of species abundances and community structure was evaluated with multiple regression. The interactive stepwise procedure included both forward selection and backward elimination (Wilkinson 1990) to select the two best models with less than five predictor variables based on the highest overall significance and r^2 values, the lowest residual variance, and normality of residual plots. Simpler models were selected over more complex models when the amount of explained variance was not significantly less with the reduced model based on an F-test comparison of the residual variances associated with the two models (Neter et al. 1983).

The importance of species interactions was estimated using contingency and correlation analysis to examine the distribution of species co-occurrences among sites. Contingency analysis was based on a two by two contingency table approach and was therefore limited to presence–absence data; correlations were based on log-transformed abundances. For both analyses, the number of statistically significant positive and negative co-occurrences were recorded for all pairwise combinations of species ($n = 496$) captured at more than five sites to avoid bias in test statistics (Zar 1974). Fisher's exact test ($p < 0.01$) and product–moment correlations ($p < 0.10$, Bonferroni corrected) were calculated to measure statistical significance. Basing the overall importance of species interactions on the total number of co-occurrences that were positive, negative, or neutral (Tonn et al. 1990) was deemed more appropriate than strict hypothesis tests for each species pair because statistical independence assumptions are violated with the latter approach (Bowers and Brown 1982).

Results

Environmental conditions

Differences in environmental conditions between sites from different regions and habitat types were larger and more

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Table 1. Environmental differences between site types that had significant differences ($p \leq 0.05$) between means in ANOVA.

(A) Habitat differences.

	Wetlands		Beaches		
Variable	Mean	Range	Mean	Range	p
Macrophyte cover (%)	36.3	0–76	5.1	0–39	<0.001
Substrate diversity (1–7)	5.3	3–7	2.0	1–5	<0.001
No. of macrophyte species	10.5	0–18	1.6	0–8	<0.001
Macrophyte diversity (0–1)	0.23	0.00–0.74	0.02	0.00–0.17	0.002
Macrophyte GFD (1–7)*	5.5	1–7	2.6	1–7	0.006
Waves (cm)	3.2	1–7	7.4	1–16	0.008
Organic carbon (%)	2.7	0.7–10.1	0.9	0.4–1.7	0.041

(B) Development differences.

	Undeveloped		Developed		
Variable	Mean	Range	Mean	Range	p
Development index (1–4)	1.5	1–2	3.2	2–4	<0.001
Macrophyte cover (%)	26.8	0–76	14.6	0–66	0.025
Conductivity (µS/cm)	273	220–374	297	248–386	0.042

(C) Regional differences.

	Lower Bay		Middle Bay		Upper Bay		
	Variable	Mean	Range	Mean	Range	Mean	p**
Latitude	44.6*a*	44.5–44.7	45.0*b*	44.8–45.1	45.8*c*	45.7–45.9	<0.001
Turbidity (JTU)	104*a*	86–137	32*b*	20–72	16*c*	9–26	<0.001
Conductivity	336*a*	292–386	257*b*	242–290	262*b*	220–316	<0.001
Nearest highway (km)	2.7*ab*	0.8–4.4	6.4*a*	2.4–12.3	1.4*b*	0.1–2.2	0.002
Macrophyte cover (%)	14*a*	0–42	12*a*	0–31	32*b*	0–76	0.003
Substrate diversity	4.3*a*	1–7	2.4*b*	1–6	4.3*a*	1–7	0.018
Macrophyte diversity	0.06*a*	0.00–0.26	0.07*a*	0.00–0.18	0.25*b*	0.00–0.74	0.020
pH	8.0*a*	7.6–8.4	7.7*b*	7.4–8.2	7.6*b*	7.2–8.0	0.027

(D) Habitat × development differences.

	Undeveloped wetlands		Developed wetlands		Undeveloped beaches		Developed beaches		
Variable	Mean	Range	Mean	Range	Mean	Range	Mean	Range	p**
Development index	1.7*a*	1–2	3.2*b*	3–4	1.3*a*	1–2	3.2*b*	2–4	0.001
Substrate diversity	5.0*a*	3.0–6.0	5.5*a*	3.0–7.0	2.0*b*	1.0–5.0	2.0*b*	1.0–3.0	0.003
Macrophyte GFD (1–7)*	6.0*a*	5.0–7.0	5.0*ab*	1.0–7.0	2.8*b*	1.0–7.0	2.4*b*	1.0–5.0	0.020
Macrophyte cover (%)	45.5*a*	24–76	27.2*b*	0–66	8.2*c*	0.39	2.0*c*	0–11	0.020
No. of macrophyte species	13.7*a*	10.0–18.0	7.3*a*	0.0–15.0	2.2*b*	0.0–8.8	1.0*b*	0.0–4.0	0.030
Waves (cm)	3.3*a*	1.0–7.0	3.0*ab*	1.0–6.0	8.0*bc*	1.0–16.0	6.8*c*	4.0–15.0	0.046
Macrophyte diversity	0.32*a*	0.14–0.74	0.15*b*	0.00–0.58	0.03*b*	0.00–0.17	0.00*b*	0.00–0.02	0.048

Note: Means followed by different letters are significantly different ($p \leq 0.05$); *GFD, growth form diversity; ** maximum significance value from ANOVA associated with differences between means.

statistically significant than between undeveloped and developed sites (Table 1). Physicochemical conditions differed greatly between regions; turbidity decreased from south to north (Table 1C) reflecting the strong trophic gradient and phytoplankton-induced turbidity in the highly eutrophic southern part of the bay (Richman et al. 1984; Sager and Richman 1991). Conductivity, pH, and water temperature exhibited similar but weaker gradients and were positively correlated with each other and turbidity ($r \geq 0.5$ for all combinations except water temperature with conductivity, $r = 0.2$). Macrophyte cover and diversity were highest in the north where mesotrophic conditions result in greater water clarity (Harris et al. 1991). Compared with beaches, wetlands generally had more diverse macrophyte communities, more areal coverage in macrophytes, more diverse substrates, higher sediment organic carbon, and smaller waves (Table 1A). These differences result from the more protected nature of wetland sites. Wetlands develop in locations less influenced by storms and currents (Geis 1979; Herdendorf 1990). Compared with developed sites, undeveloped sites tended to have more macrophyte cover and lower conductivity (Table 1B). Undeveloped wetlands had the highest macrophyte diversity and cover, while developed beaches had the lowest Table 1D).

Patterns among sites in species space

Over 40 000, primarily immature (>90%), fish from 54 species

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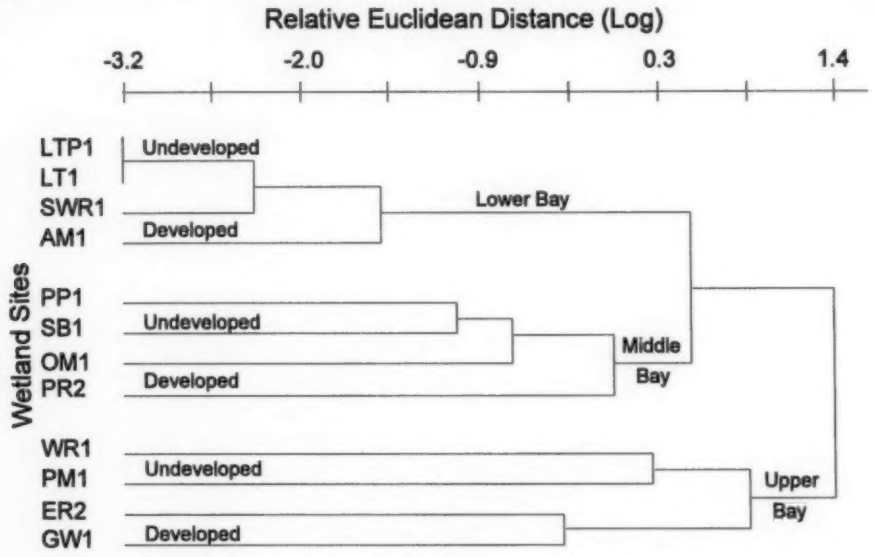
Fig. 2. Cluster analysis (Ward's method) of wetland sites in species space (text annotations included to aid in cluster interpretations; site codes detailed in Appendix).



and 20 families were captured during the study (see Brazner 1994, 1997 for details); thus, data reflect primarily the ecology of young individuals. To simplify the process of discerning patterns, initial analyses were conducted for wetland sites only. Cluster analysis of these data revealed distinct clusters among sites from different regions and sharp differentiation associated with development status (Fig. 2). Ordination also revealed strong separation between regions along axis 1, with some separation between developed and undeveloped sites along axis 2 (Fig. 3). These two axes accounted for 88% of the original distances calculated among sites. In addition, some of the variation along axes 1 and 3 apparently was related to exposure of the sites; wave height was one of the most highly correlated abiotic factors with these axes ($r_{\text{axis }1} = -0.80$, $r_{\text{axis }3} = -0.66$). Sites that scored lowest along axes 1 and 3 tended to be the more exposed wetland sites (e.g., AM1, SWR1, OM1; see Table 2 for site codes) while those that scored higher on these axes were among the most protected sites (e.g., PM1, PR2, WR1).

Differentiating patterns became more complicated when all sites were included, but some of the same trends were apparent. A strong regional signal was still discernable in the ordination of these data (Fig. 4). Regional separation occurred primarily along axis 1. This axis accounted for 50% of the original distances among sites of the 83% accounted for by the first three axes together. Gizzard shad *(Dorosoma cepedianum)*, emerald shiners (*Notropis atherinoides*), spotfin shiners (*N. spilopterus*), white bass (*Morone chrysops*), and yellow perch *(Perca flavescens)* were the fish species that were most closely correlated with axis 1 (Table 3). Except for yellow perch, which was caught consistently throughout the bay (especially at wetland sites), these species were nearly always most abundant in the lower bay and could be characterized as lower bay species. The environmental variables that were most highly correlated with axis 1 were turbidity, latitude, water temperature, conductivity, and pH (Table 3).

Separation among sites based on habitat differences was also apparent but weaker than the regional differentiation. Middle and upper bay sites had the most distinct separation between beaches and wetlands (Fig. 4). Separation among the different types of sites was the weakest in the lower bay. Habitat separation among sites was primarily along the third axis with most sites (9 of 11) scoring less than 0.4 as wetlands and most sites (10 of 13) with scores greater than 0.4 as beaches. This axis accounted for 23% of the original distances calculated between sites and was most closely correlated with pumpkinseeds (*Lepomis gibbosus*), largemouth bass (*Micropterus salmoides*), golden shiners *(Notemigonus chrysoleucas)*, johnny darters (*Etheostoma nigrum*), and bowfin (*Amia calva*) (Table 3). These fishes are indicative of wetlands except for johnny darters, which had a significant beach component to their catch (Table 2). However, 84% of johnny darters from beaches came from the upper bay beach site (WR2) that ordinated closest to wetland sites. The environmental variables that correlated most closely to axis 3 were the four macrophyte-related variables and wave height (Table 3). This suggests that the diversity and physical structure of the macrophyte community influenced the fish assemblages. The negative correlation with wave height is consistent with the positive correlation with macrophytes (Geis 1979, 1985; Keddy 1983).

Separation between sites based on development was weaker than separation between habitat or region. However, some separation was apparent along axis 2 which accounted for 10% of the original distances between sites (Fig. 4). Separation associated with development was more nonlinear than regional or habitat associated separation, but within regional clusters of sites, development-associated separation was fairly clear. The fish species that were most closely correlated with axis 2 were smallmouth bass, common shiner (*Luxilus cornutus*), alewife (*Alosa pseudoharengus*), rock bass (*Ambloplites rupestris*), and spotfin shiner (Table 3). The positively correlated smallmouth bass were more prevalent (76% of all captured) at

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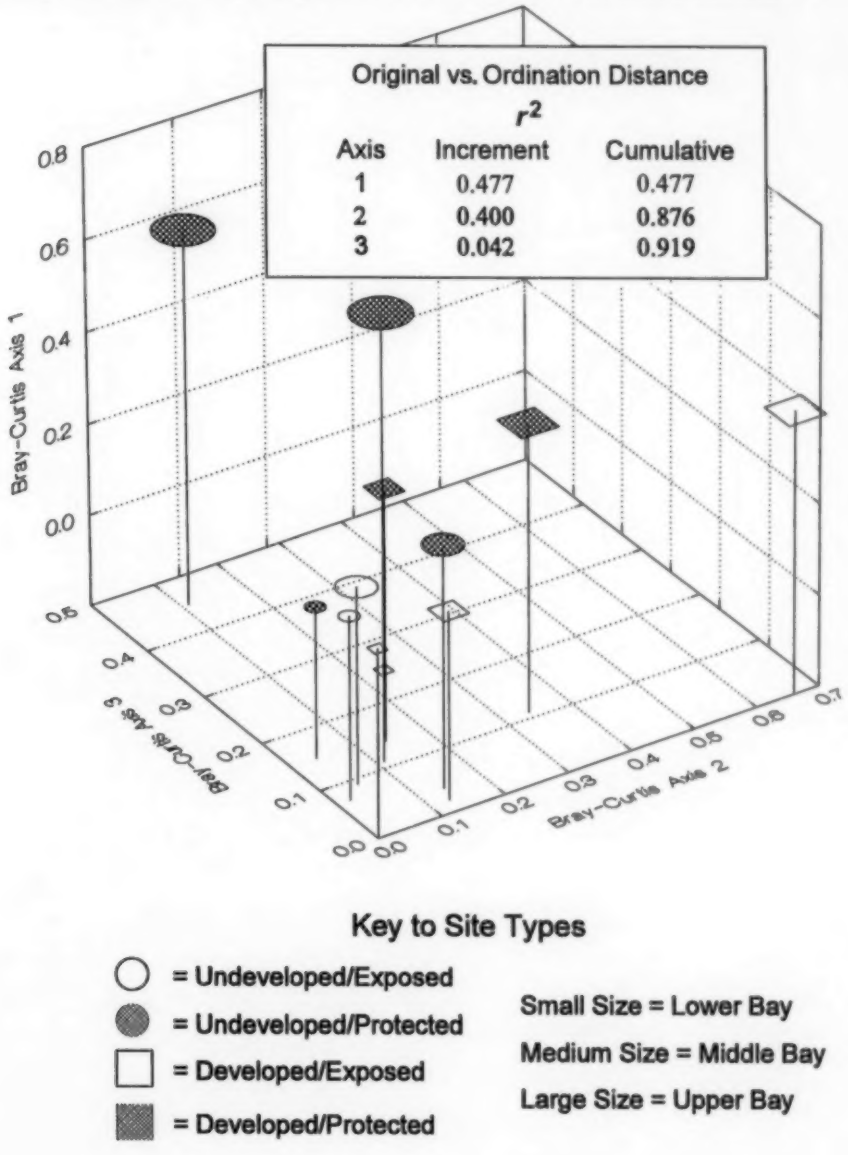
Fig. 3. Bray–Curtis ordination (variance-regression endpoint selection) on wetland sites in species space (correlations between site distances along each of the ordination axes and distances in the original n-dimensional data matrix space tabled in inset box).



developed sites, while the negatively correlated spotfin shiners were more prevalent (70% of all captured) at undeveloped sites. Environmental factors most closely correlated with axis 2 were distance to the nearest highway, turbidity, latitude, water temperature, and wind speed (Table 3). This suggests that a combination of regional and development-related factors influenced patterns along this axis.

Discriminant analyses helped quantify the statistical distinctness of the ordination patterns. Using the eight species with the strongest regional signals (e.g., highest correlations with axis 1) in the ordinations (Table 3), discriminant analysis classified 23 of 24 sites correctly (Table 4). Only one middle bay site (SB2), was misclassified as an upper bay site. This misclassification is consistent with ordination results where SB2 clustered closely with developed sites from the upper bay (Fig. 4). Discriminant analysis using species with strong habitat affiliations in the ordinations classified only 18 of 24 sites correctly (Table 4). All but 1 (92%) of the wetland sites and 7 of 12 (58%) beach sites were classified correctly. The middle bay wetland, PP1, was the only wetland misclassified, but all lower bay beaches and the upper bay beach, WR2, were misclassified as wetlands. WR2 also mapped closest to wetlands in the ordination (Fig. 4). These results suggest that wetland fish assemblages were more distinctive than beach assemblages

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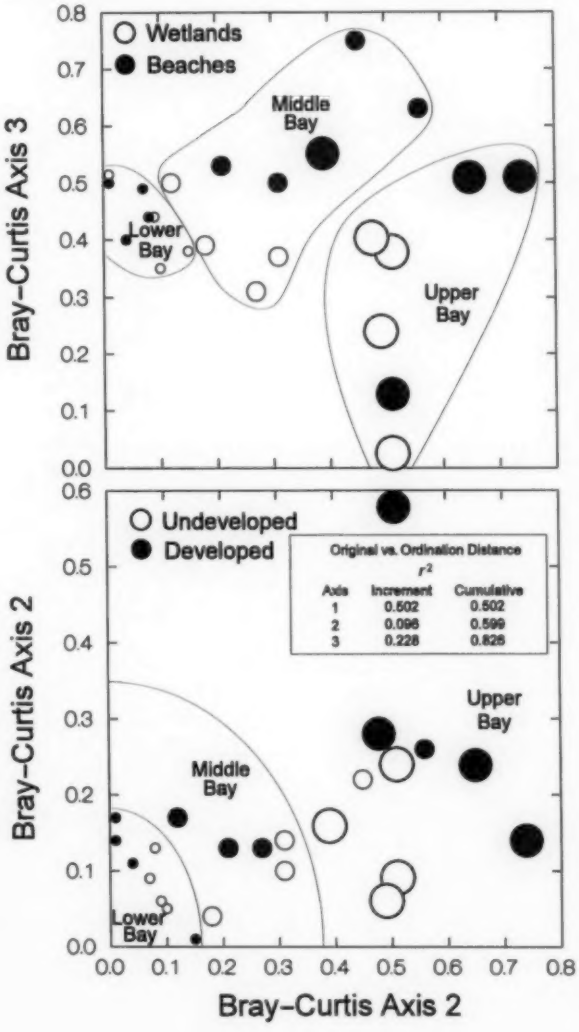
Table 2. Fish species and environmental variables most closely correlated (r) with Bray–Curtis ordination axes 1–3.

	Axis 1	Axis 2	Axis 3
Fish species			
Gizard shad	–0.854		
Emerald shiner	–0.812		
Spotfin shiner	–0.807	–0.537	
White bass	–0.798		
Yellow perch	–0.742		
Smallmouth bass		0.672	
Common shiner		–0.565	
Alewife		–0.539	
Rock bass		0.521	
Pumpkinseed			–0.806
Largemouth bass			–0.732
Golden shiner			–0.708
Johnny darter			0.704
Bowfin			–0.680
Environmental variables			
Turbidity	–0.915	–0.558	
Latitude	0.893	0.482	
Water temperature	–0.609	–0.445	
Conductivity	–0.601		
pH	–0.577		
Highway distance		–0.596	
Wind speed		–0.273	
% Macrophyte cover			–0.793
Macrophyte growth form diversity			–0.714
Macrophyte diversity			–0.710
Macrophyte species richness			–0.691
Waves			0.670

across the regional and developmental range of sites sampled in the bay. The discriminant analysis using species most closely associated with development-related axes in the ordinations classified only 16 of 24 (67%) of the sites correctly. Differences in fish assemblages resulting from development were apparently weak, at least when considered irrespective of habitat. However, discriminant analysis using species most closely correlated with both habitat and development-related axes (axes 2 and 3) provided additional insight. Here, the overall correct classification rate was poor (58%), but all undeveloped wetland sites were classified correctly.

Cluster analysis of species in site space resulted in seven main groups that were separated by overall abundance and particular site affinities (Fig. 5). Species highest on the dendogram were among the most abundant (e.g., spottail shiners, *N. hudsonius*, yellow perch) while those near the bottom were rarer. Distinct groups of species were abundant in the lower bay (e.g., emerald shiner, gizzard shad, white bass), or common in the upper bay (e.g., sand shiner, *N. stramineus*, white sucker, *Catostomus commersoni*, smallmouth bass). Species typical of undeveloped wetlands in the upper bay clustered into two groups, differing in abundance: commonly captured fishes (e.g., golden shiners, pumpkinseeds, and largemouth bass) and less common species (bowfin and walleye, *Stizostedion vitreum*). Several species typically captured at developed sites also clustered together but were separated by lower bay (e.g., freshwater drum, *Aplodinotus grunniens*, quillback, *Carpiodes cyprinus*, channel catfish, *Ictalurus punctatus*) or

Fig. 4. Bray–Curtis ordination (variance-regression endpoint selection) on all sites in species space (smallest circles, lower bay sites, medium-sized circles, middle bay sites, largest circles, upper bay sites; lines surrounding and separating groups are aids for pattern interpretation but not statistically based; correlations between distances along each of the ordination axes and distances in the original n-dimensional data matrix space appear as inset box in lower plot).



middle bay (e.g., yellow bullhead, *Ictalurus natalis*, trout-perch, *Percopsis omiscomaycus*, rainbow smelt, *Osmerus mordax*) affiliations.

Species co-occurrence analyses suggested that biotic interactions, such as competition and predation, were unimportant. The summary of species co-occurrences obtained from both contingency and correlation analysis revealed that approximately 95% of the 496 possible species co-occurrence distributions were neutral (Table 5). Both analyses produced similar and nearly equal numbers of positive co-occurrences (17 for contingency and 18 for correlation analysis) and fewer and

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different numbers of negative co-occurrences (10 for contingency and 2 for correlation analysis). Species with significant positive co-occurences all had strong affinities for a particular region, usually the lower bay. Nearly all negative correlations could be explained by the strong differences in regional distributions. One exception might be the rock bass – alewife relationship identified with correlation analysis. Rock bass and alewife occurred in the same types of sites in both the middle and upper bay, but their abundances were negatively related ($r = -0.78$), indicating the possibility of a competitive or predator–prey relationship.

The influence of several abiotic factors on species distribution patterns was evident through ordinations of species directly in environmental space. The first three axes accounted for 99% of the original distance calculated among sites; 83% with axis 1, 13% with axis 2, and 3% with axis 3. Based on the correlations of environmental variables with each ordination axis, species locations along axis 1 were driven by regional factors, along axis 2 by habitat, and along axis 3 by development. Latitude, turbidity, and pH were most closely correlated with axis 1 ($r > 0.95$); macrophyte diversity, substrate diversity, and wave height were most highly correlated with axis 2 ($r > 0.80$); and the development index, conductivity, and macrophyte growth form diversity were most closely correlated with axis 3 ($r \geq 0.60$).

Six of the environmental variables closely correlated with ordination axes of species in environmental space had particularly clear gradients. Contour overlays revealed marked separation primarily along axis 1 among three species groups corresponding to their scores (for details see Brazner 1994) for latitude and turbidity (Figs. 6E and 6F). Species positioned low along axis 1 were usually from lower bay sites with high turbidity, while species positioned high along axis 1 were usually from upper bay sites with low turbidity. Thus, the three species groups were typical of lower, middle, and upper bay assemblages. Contour overlays for other factors that varied on a regional scale, such as conductivity, pH, and water temperature, were similar to those for turbidity and latitude, but separation among species groups was less distinct.

Contour overlays also revealed clear separation primarily along axis 2 among three species groups corresponding to their scores for substrate diversity (Fig. 6D). Species positioned highest along axis 2 were usually from sites of low substrate diversity (beaches) while species positioned lowest along this axis included typical wetland fishes. The response to sediment organic carbon was similar but not as clear as the response to substrate diversity. Another important gradient related to habitat was the macrophyte gradient. Contour overlays for number of macrophyte species, growth form diversity, and areal coverage all revealed clear separation among fish along both axes 1 and 2. The clearest response was apparent for the combined macrophyte diversity index, which divided fishes into four groups (Fig. 6C). Fish from sites with the highest macrophyte diversity (undeveloped wetlands) were positioned in the lower right hand corner of the plot, while those in the upper left hand corner were usually from sites with little or no macrophyte cover (developed beaches). Contours for wave height were similar to those for macrophyte diversity in that there was separation along axes 1 and 2 (Fig. 6B). However, separation along axis 3 also was observed when plotted against axis 2 suggesting that wave height is related to a variety of factors

Table 3. Site classification matrices based on discriminant analyses with eight fishes that were most closely correlated (the four highest **positive** and four highest negative r values) with Bray–Curtis ordination.

(A) Axis 1 (regional discrimination).

	Predicted region				
Actual region	Lower	Middle	Upper	% Correct	Species
Lower	8	0	0	100	GZ, ES, WB
Middle	0	7	1	88	SF, **RB**, **SB**
Upper	0	0	8	100	**LP**, **SS**
Total	8	7	9		

(B) Axis 2 (development discrimination).

Actual	Predicted development			
development	Undeveloped	Developed	% Correct	Species
Undeveloped	8	4	67	AW, SF, GZ, CS
Developed	4	8	67	**SB**, **RB**, **SS**, **TP**
Total	12	12		

(C) Axis 3 (habitat discrimination).

	Predicted habitat			
Actual habitat	Wetland	Beach	% Correct	Species
Wetland	11	1	92	GS, PS, JD, BF
Beach	5	7	58	**AW**, **RS**, **TP**, **ST**
Total	16	8		

(D) Axes 2 and 3 (habitat × development discrimination).

	Predicted site type					
Actual site type	UW	DW	UB	DB	% Correct	Species
UW	6	0	0	0	100	GS, PS, **AW**
DW	1	2	1	2	33	**TP**, **SB**, **RB**
UB	1	2	2	1	33	SF, CS
DB	0	1	1	4	67	
Total	8	5	4	7		

Note: Two-letter species codes are given in the Appendix.
UW, undeveloped wetland; DW, developed wetland; UB, undeveloped beach; DB, developed beach.

(e.g., regional, habitat, development). A gradient in fish response was clearly apparent for one development-related variable, the development index. Contours of species scores for the development index were most clearly separated along axes 1 and 3 (Fig. 6A). Species usually from sites with the highest development index (e.g., channel catfish, quillback, green sunfish) were in the upper left hand corner of this plot, while those typifying undeveloped sites (e.g., johnny darter, bowfin, largemouth bass) were in the lower right portion of the plot. Contours of conductivity and slope along these same axes provided similar but less distinct separation.

Regression analysis provided a more predictive and quantitative approach for understanding which abiotic factors were driving a limited number of species distributions (the five most abundant fishes and five other species of special ecological interest in Green Bay), as well as patterns in species richness and overall fish abundance. The two best models for each fish or community structure variable (Table 6) were used to evaluate which environmental factors were most predictive of fish species abundance and assemblage structure. Only two models

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Table 4. Summary of species co-occurrences based on 2 × 2 contingency analysis of presence–absence data and correlation analysis of total abundance data for all species captured at more than five sites.

Analytical technique	Positive co-occurrences	Negative co-occurrences	Neutral
Contingency analysis	17	10	469
(χ^2, $p \leq 0.01$)	3.4%	2.0%	94.6%
	White bass (SF, GZ, GR, CP, FD, QB)	Sand shiner (GR, GZ, CP, FD)	
	Green sunfish (GZ, SF, CS, WB, FD)	Golden shiner (TP, RS)	
	Gizzard shad (WB, GR, CP, FD)	Rock bass (CS, GR)	
	Freshwater drum (GZ, WB, GR, CP)	Smallmouth bass (CP, GZ)	
	Common carp (GZ, WB, FM, FD)	Gizzard shad (SS, SB)	
		Common carp (SS, SB)	
		Green sunfish (SS, RB)	
Correlation analysis	18	2	476
(r, $p \leq 0.10$, Bonferroni corrected)	3.6%	0.4%	96.0%
	Gizzard shad (YP, ES, WB, CP, FD)	Rock bass (AW, SF)	
	White bass (GZ, ES, CP, FD)		
	Freshwater drum (GZ, ES, WB)		
	Emerald shiner (GZ, WB, FD)		
	Golden shiner (PS, BC)		
	Logperch (JD, LB)		

Note: Species with the greatest number of significant co-occurrences have specific pairwise combinations listed separately. Two-letter species codes are given in the Appendix.

were single factor regressions; macrophyte cover was highly predictive of bluegill abundance and macrophyte diversity was highly predictive of golden shiner abundance. Of the remaining 22 models, 13 required only 2 input variables, while 7 required 3 input variables, and 2 had 4 input variables. The most predictive variables included both regional and habitat related factors. Turbidity, latitude, or water temperature was the most explanatory variable in half of the 24 models, while one of the macrophyte variables was most explanatory in 10 of the remaining 12 models. Development variables had high predictive power in only three models: one white sucker model and the two models of overall abundance.

Discussion

Distinct fish assemblages were present in the shallow inshore habitats of Green Bay. The greatest differentiation among assemblages was observed at a regional scale, but there also were differences among assemblages from different habitats. Differences due to development were least apparent, although assemblages at undeveloped wetlands were unique from those at other habitat-by-development site combinations. In addition, assemblages from developed and undeveloped sites in the upper bay were relatively distinct, as were differences between very highly developed sites (e.g., AM1, BB1) and all other sites.

Regional patterns

Site separation within regions was the smallest in the lower bay and largest in the upper bay. This may reflect the general level of degradation and mixing in each region. The lower bay is an Area of Concern (IJC 1987) with a large contaminant load, hypereutrophic nutrient status, high turbidity, and intense development on the shoreline throughout much of the area. The upper bay has lower population density, lower trophic status, clearer water, and less human development (Bertrand et al. 1976). The lower bay is also well mixed because of its shallow, open morphometry; approximately 70% of the water inside of Long Tail Point and Point Sable is comprised of Fox River water (Epstein et al. 1974). This results in a high degree of physical and chemical similarity throughout the lower bay and may be the reason fish assemblages are most similar there. In the upper bay, where turbidity is low and mixing is presumably less because of the protected nature of Little Bay de Noc, there was greater differentiation among sites and the influence of development was more apparent. These results are also consistent with the finding that infertile wetlands are more sensitive to human disturbance and have greater variation in vegetation than wetlands in fertile areas (Moore et al. 1989).

Environmental influences

Of all environmental factors, turbidity had the most influence on fish assemblage structure. Turbidity varied primarily on a regional scale and reflected the trophic gradient. About half of the turbidity is biotic, while the other half results from erosion, runoff, and resuspension of sediments in the shallow lower bay (Harris et al. 1991; Millard and Sager 1994). Other factors that covary with turbidity also reflect (e.g., conductivity, pH) or contribute to (e.g., water temperature) the trophic gradient. They each may have independently influenced fish assemblage structure, but turbidity was correlated most consistently with the major axes of variation in the sites in species space ordinations, was most predictive in the regression equations, and had clearly separated contours in ordinations of species in environmental space.

Turbidity has also been implicated as a primary factor limiting macrophyte communities in the Great Lakes (Phillips et al. 1978; Stuckey 1989; Crowder and Painter 1991) and in the lower and middle sections of Green Bay (Harris et al. 1991; McAllister 1991). This is especially relevant to this study because, after turbidity, macrophytes were the next most important factor structuring the fish assemblages. Macrophyte cover was correlated with assemblage differences between wetlands

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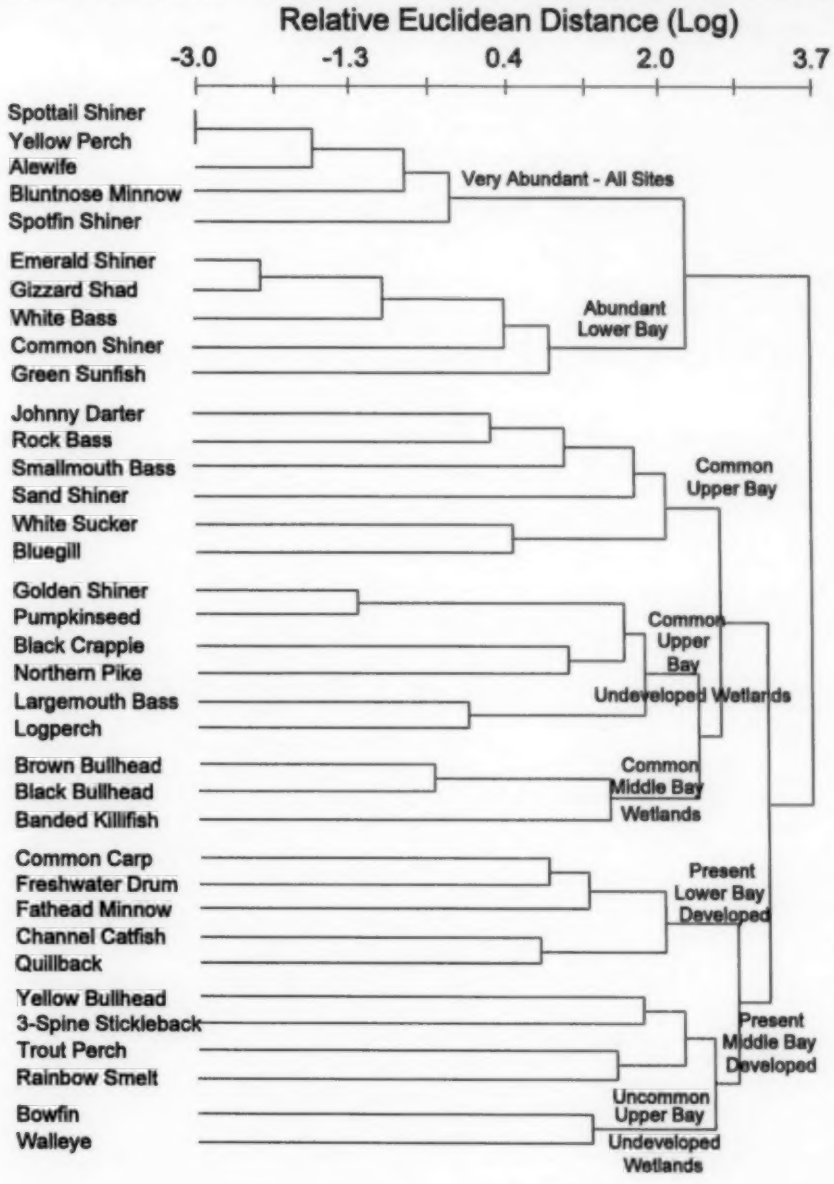
Fig. 5. Cluster analysis (Ward's method) of species in site space using all sites (text annotations included to aid in cluster interpretations).



and beaches and between undeveloped and developed wetlands. Where macrophyte cover and richness was high, fish species richness and abundance also tended to be high. This same pattern was apparent in other Great Lakes littoral habitats (Minns et al. 1994) and in more inland lakes (Keast et al. 1978; Benson and Magnuson 1992); it is driven by the fact that fish are attracted to the increased habitat heterogeneity that macrophytes provide (Tonn and Magnison 1982; Eadie and Keast 1984; Robinson and Tonn 1989) and the invertebrate prey species that utilize macrophytes for substrates and food (Keast and Harker 1977; Keast 1978; Wiley et al.1984). However, extremely high densities of macrophytes (>70–80% cover) can limit their utility to fish and result in lower overall fish species richness than in moderately vegetated habitats (Crowder and Cooper 1982; Killgore et al. 1989; Lillie and Budd 1992). Macrophytes rarely reached high densities during this study, but heavy macrophyte cover is not uncommon in other Great Lakes coastal wetlands and may become more common in Green Bay as the zebra mussel (*Dreissena polymorpha*) invasion advances and leads to greater water clarity and better growing conditions for macrophytes (Skubinna et al. 1995). A better characterization of the relationship between fish distribution and macrophyte community structure would be useful, since a relationship between areal coverage of vegetated

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Table 5. Regression relationships between environmental variables and individual fish species or community structure variables measured at each site.

Species or community component	Best models	r	p
Yellow perch	**44.03** – 0.94(latitude) + 0.18(substrate diversity)	0.80	<0.001
	–0.63 + 1.47 log(turbidity) + 0.14(macrophyte GFD)	0.78	<0.001
Spottail shiner	29.31 – 0.86(latitude) + 8.53 log(water temperature)	0.70	0.001
	–12.48 + 9.59 log(water temperature) + 0.97 log(turbidity)	0.66	0.002
Alewife	**29.62** + 2.39 log(turbidity) – 0.03(% macrophyte cover) – 12.81log(conductivity) + 0.23(% sed. OC)	0.83	<0.001
	18.67 + 1.77 log(turbidity) – 0.02(% macrophyte cover) – 7.89 log(conductivity)	0.76	0.001
Spotfin shiner	**45.73** – 1.32(latitude) + 10.88 log(water temperature) + 0.06(city distance)	0.93	<0.001
	47.80 – 1.39(latitude) + 12.13 log(water temperature)	0.91	<0.001
Bluntnose minnow	**37.48** + 0.49(macrophyte GFD) – 0.83(latitude) – 0.97(no. of macrophyte species)	0.85	<0.001
	32.95 + 0.28(macrophyte GFD) – 0.72 log(latitude)	0.81	<0.001
Bluegill	–0.10 + 0.02(% macrophyte cover) + 0.11(% slope)	0.76	<0.001
	0.19 + 0.02(% macrophyte cover)	0.71	<0.001
Northern pike	**0.30** + 2.15(macrophyte diversity) – 0.12(% sed. OC) – 0.05(% slope)	0.81	<0.001
	0.16 + 1.76(macrophyte diversity) – 0.11(% sed. OC)	0.76	<0.001
Golden shiner	0.11 + 3.43(macrophyte diversity) – 0.11(% sed. OC)	0.87	<0.001
	0.01 + 2.59(macrophyte diversity)	0.83	<0.001
White sucker	**–32.85** + 2.24 log(wind speed) + 0.73(latitude) + 0.07 log (city distance)	0.75	0.001
	0.15 + 1.96 log(wind speed) + 0.15(% slope)	0.64	0.004
Sand shiner	–42.09 – 2.01 log(no. of macrophyte species) + 0.94(latitude) + 0.42(macrophyte GFD) – 0.17(% slope)	0.85	<0.001
	–40.07 – 0.49 log(no. of macrophyte species) + 0.91(latitude)	0.64	0.004
No. of species	2.06 + 8.65 log(turbidity) + 1.05(macrophyte GFD)	0.79	<0.001
	5.71 + 7.66 log(turbidity) + 4.06 log(no. of macrophyte species)	0.77	<0.001
Log total abundance	**–4.39** + 5.17 log(water temperature) + 0.54 log(turbidity) – 0.17(development index)	0.82	<0.001
	1.81 + 0.85 log(turbidity) – 0.14(development index) + 0.06(macrophyte GFD)	0.78	<0.001

Note: The two best models with less than four variables and all coefficients significantly different from zero, $p \leq 0.10$, are presented. Models were selected through interactive stepwise regression procedure; those with the highest r, overall significance values, lowest residual variance, and normal residual plots were deemed "best." Simpler models were always selected over more complex models when all else was equal. Constants given in boldface are significantly different from zero. GFD, growth form diversity; sed. OC, sediment organic carbon.

wetland habitat and open lake fisheries would be important to quantify. This sort of relationship has been observed in coastal marine ecosystems (Turner 1977; Boesch and Turner 1984; Deegan 1993) but is not well established in Great Lakes coastal systems even though recent studies suggest a wetland–fisheries connection may exist. (Whillans 1989; Keough et al. 1996). Studies designed to further examine macrophyte – littoral fish – open lake fish community interactions are currently in progress on both Lake Superior and Lake Michigan (D.A. Wilcox, National Biological Service, Ann Arbor, Mich., personal communication; J.C. Brazner, unpublished data). Indications are that there may be a significant relationship that is dependent upon the morphometry and configuration of littoral habitats in a particular area (e.g., coastal wetland hydrogeomorphic type).

Macrophyte communities in the Great Lakes are structured by a variety of factors in addition to turbidity (Stuckey 1989). Water level fluctuation is clearly one of the most important (Keddy and Reznicek 1985; Keough 1992; Wilcox and Meeker 1992), and exposure to wind and wave energy is another (Keddy 1982). Exposure also is one of the more obvious physical differences between wetlands and beaches and may be the underlying reason why substrate diversity and sediment organic carbon were closely related to differences in fish assemblage structure between these site types. Organic carbon and particle diversity in sediments reflect differences in exposure (Cole and Weigmann 1983; Wilson and Keddy 1985) and can affect macrophyte community structure, colonization, and growth (Keddy 1983; Wilson and Keddy 1985). More protected sites, less subject to storm, current, and wave action, have more organic carbon and higher percent silt and clay in their sediments. It appears that intermediate exposure levels result in the highest macrophyte species richness (Keddy 1983) and, based on this study, also the highest fish species richness.

Quantifying development

Although some differences in the fish assemblages were attributed to human development, particularly when only wetland site data were included in the analysis, effects of development were surprisingly weak. The semiquantitative index of development was the most useful, while distance to the nearest city and highway were poorer predictors. A better index of disturbance from different types of development is needed. Because much of the Great Lakes shoreline is fringed with roads and cities, distances to roads and cities may have been too similar among sites to represent development effectively. Population density, road density, or percentages of land in various uses near a site quantified using a geographic information system approach (e.g., Detenbeck et al. 1993; Weaver and Garman 1994) might have been more effective. This approach has been used particularly effectively to compare differences in the fish community-based Index of Biotic Integrity with varying watershed conditions in Ontario streams (Steedman 1988).

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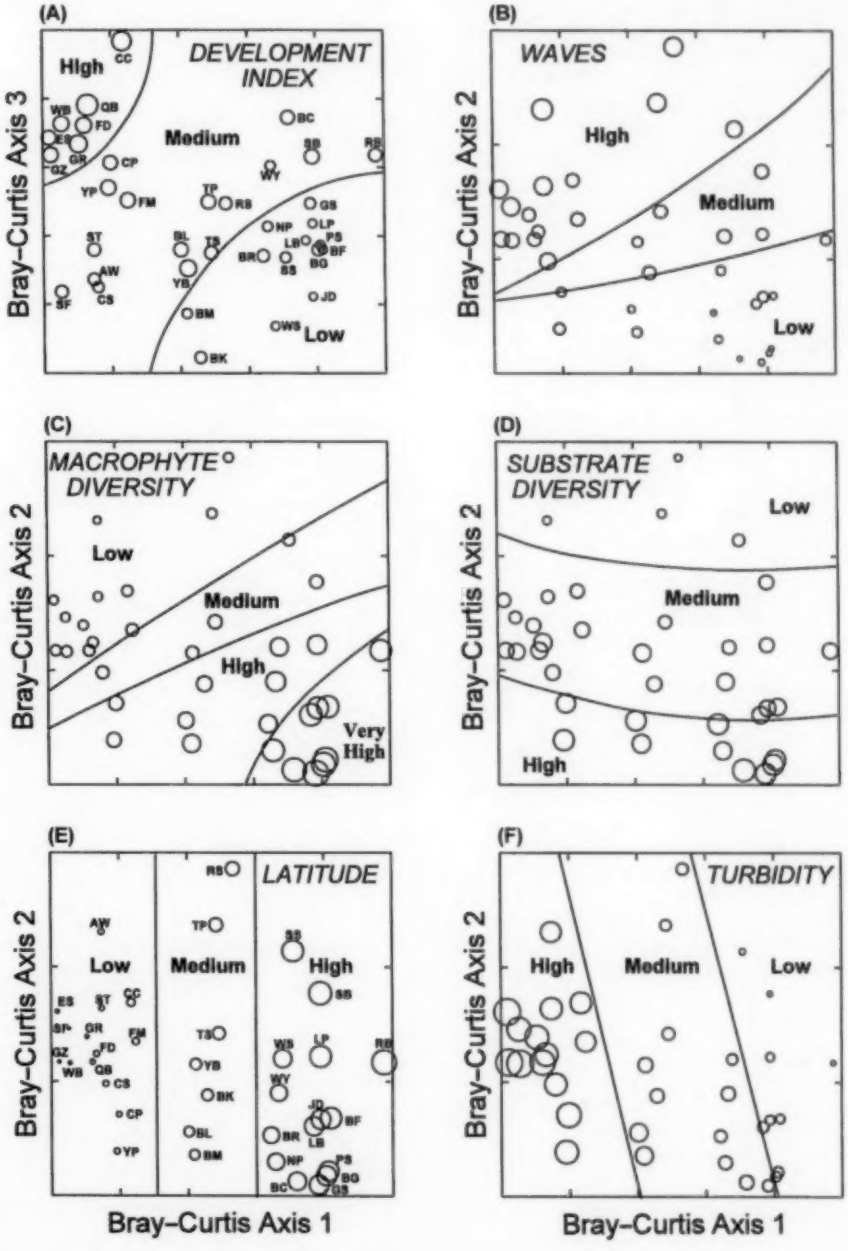
Fig. 6. Bray–Curtis ordination (variance-regression endpoint selection) of species in environmental space using all sites; contours for abiotic variables overlayed (point size based on species scores for each variable; contours are best fit lines that separate species into three or four response levels for each variable based on species scores for that variable (details in Brazner 1994). The two-letter species codes are defined in the Appendix. Note that all axis 2 versus axis 1 codes are detailed in the panel for latitude, and upper left panel is the only plot of axis 3 versus axis 1.



Biotic interactions

Despite the possible exception noted for rock bass and alewives and limitations inherent to the kinds of analyses we used (Bowers and Brown 1982; Tonn et al. 1990), strong species interactions appeared to be rare, and their direct influence on the structure and patterns in the fish assemblages was probably minimal. The apparent unimportance of biotic interactions to these fish assemblages may be related to high variability in physical conditions typical of nearshore habitats in the Great Lakes. Hourly, daily, seasonal, and annual fluctuations in

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water level along with strong storm surges, upwellings, seiche activity, wide daily temperature fluctuations, ice scour, and other harsh environmental conditions are associated with these habitats in Green Bay (Heaps et al. 1982; Miller and Saylor 1985; Lathrop et al. 1990). This variability may preclude competition and predation from becoming strong organizing forces (Wiens 1984; Menge and Sutherland 1987; Winemiller and Rose 1992). Other studies of intertidal communities (Sousa 1979), coral reef fishes (Sale 1980), birds (Tramer 1969; Rotenberry 1978), and phytoplankton (Tramer 1969) from unpredictable environments support this hypothesis. The fact that >90% of the fish captured during this study were immature probably also reduced the likelihood of detecting predator–prey interactions and reinforces the idea that coastal wetlands have a primary role as nursery habitats for fish (Stephenson 1990; Brazner 1997).

Overall, it appears that a combination of regional and site-specific factors were important in structuring fish assemblages in Green Bay's coastal wetland and beach habitats. Tonn et al. (1990) refer to this as the "balance between local and regional processes" in their study of patterns in fish assemblages from small North American and Finnish lakes. Although assemblages in a particular location may be primarily the result of local processes, factors that vary across larger spatial scales, such as geomorphic conditions or climatic factors, may also have a significant influence (Ricklefs 1987). Understanding scale of analysis in the interpretation of patterns may be especially important for littoral zone organisms. It has been suggested that the high complexity of littoral environments, compared with more homogeneous pelagic habitats, requires the ecology of littoral organisms be studied across a hierarchy of influences that encompass both regional and site-specific scales (Duarte and Kalff 1990). After completing this sort of analysis in Green Bay, it appears that regionally varying factors (e.g., turbidity) had the most influence on littoral fish assemblages, and undeveloped wetlands provide the most valuable and intensely utilized fish habitat, particularly as nursery areas. These results and studies of fishes associated with vegetated and beach habitats in smaller inland lakes (Guillory et al. 1979; Bryan 1992) and altered and unaltered littoral habitats from the Great Lakes (Poe et al. 1986; Johnson 1989) support the idea that a broad geographic perspective is important and that wetland habitats should be given special consideration in ecosystem management plans for the Great Lakes.

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Appendix. Number of individuals of each species captured by site (reduced data matrix only, which includes the 36 most abundant species of 54 captured.

Site, code, and development*	YP	ST	AW	SF	BM	GZ	WS	ES	CS	BG	WB	SS	GR	GS
Wetlands														
Lower Bay														
Long Tail Point Wetland, LTP1, U	3525	789	265	194	285	384	14	113	105	18	52	0	3	1
Little Tail Point Wetland, LT1, U	942	207	142	227	116	264	10	31	34	3	226	0	5	0
Atkinson Marsh, AM1, D	333	224	92	45	15	160	1	251	6	0	106	0	2	0
Sensiba Wildlife Refuge, SWR1, D	1108	241	124	220	259	137	103	224	81	6	9	0	2	3
Middle Bay														
Pecor Point Marsh, PP1, U	170	316	151	436	23	13	297	24	174	4	9	0	5	1
Seagull Bar Wetland, SB1, U	267	513	193	82	607	1	53	4	7	42	0	0	24	0
Oconto Marsh, OM1, D	919	985	80	53	544	4	2	2	277	0	0	0	6	0
Peshtigo Marsh, PR2, D	99	176	93	104	520	0	63	0	3	36	0	20	41	0
Upper Bay														
Whitefish River Mouth Wetland, WR1, U	99	0	0	0	411	0	388	0	5	327	0	0	0	182
Portage Marsh, PM1, U	20	0	37	0	5	1	8	0	0	205	0	0	0	71
Escanaba River Mouth Wetland, ER2, D	198	236	14	0	18	0	13	2	0	4	0	6	0	12
Gladstone Wetland, GW1, D	517	33	0	0	5	0	0	0	0	1	0	57	0	0
Beaches														
Lower Bay														
Long Tail Point Outer Beach, LTP3, U	312	386	86	1708	76	208	5	136	19	1	15	0	5	1
Point Sable Beach, PS1, U	695	399	74	7	27	34	13	259	8	0	56	0	193	0
Bay Beach, BB1, D	587	294	49	119	47	195	1	139	1	0	118	0	113	0
Long Tail Point Inner Beach, LTP2, D	709	191	75	250	87	514	12	151	54	0	74	0	23	6
Middle Bay														
Seagull Bar Outer Beach, SB3, U	5	106	65	4	3	0	2	2	0	2	0	0	0	0
Peshtigo River Outer Beach, PR4, U	57	242	685	330	83	0	109	1	5	0	0	75	4	0
Peshtigo River Inner Beach, PR3, D	342	2933	1136	465	39	19	30	17	7	0	1	6	0	0
Seagull Bar Inner Beach, SB2, D	29	1	49	0	0	0	4	0	0	0	0	0	4	0
Upper Bay														
Whitefish River Mouth Beach, WR2, U	141	79	1	0	534	0	83	0	1	4	0	58	0	2
Portage Point Beach, PM2, U	8	232	868	0	6	0	83	71	19	0	0	385	0	0
Escanaba River Mouth Beach, ER1, D	11	5	14	0	2	0	115	1	0	36	0	2	0	0
Escanaba River Delta Beach, FR3, D	13	2	28	1	8	0	43	0	0	1	0	18	0	0
Totals	11 106	8590	4321	4245	3720	1934	1452	1428	806	690	666	627	450	279

Note: The two-letter species codes are as follows: YP, yellow perch; ST, spottail shiner; AW, alewife; SF, spotfi shiner; BM, bluntnose minnow; GZ, gizzard shad; WS, white sucker; ES, emerald shiner; CS, common shiner; BG, bluegill; WB, white bass; SS, sand shiner; GR, green sunfish; GS, golden shiner; RB, rock bass; PS, pumpkin seed; JD, Johnny darter; BL, black bullhead; BR, brown bullhead; SB, smallmouth bass; CP; common carp; BK, banded killifish; FM, fathead minnow; LB, largemouth bass; BC, black crappie; TP, troutperch; FD, freshwater drum; LP, logperch; RS, rainbow smelt; NP, northern pike; CC, channel catfish; OB, quillback; YB, yellow bullhead; WY, walleye; BF, bowfin; TS, threespine stickeback.

*D, sites modified by human development; U, relatively undeveloped site.

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RB	PS	JD	BL	BR	SB	CP	BK	FM	LB	BC	TP	FD	LP	RS	NP	CC	QB	YB	WY	BF	TS
0	3	5	6	1	0	19	1	0	1	1	0	4	0	0	0	0	2	2	1	0	1
0	1	0	3	1	0	10	2	4	0	2	0	4	0	1	2	0	0	0	0	0	0
0	0	0	0	0	0	3	0	1	0	1	0	7	0	0	1	3	6	0	0	0	0
0	1	1	2	0	2	1	1	26	1	1	0	1	1	0	0	0	4	0	0	0	0
0	0	5	1	4	0	2	15	0	0	0	0	0	0	0	5	0	0	0	0	0	0
8	0	3	3	0	7	0	15	1	5	0	0	0	0	0	1	1	0	1	0	1	0
0	0	4	0	0	1	1	7	3	1	0	21	0	1	0	1	0	0	1	0	0	4
0	8	7	93	79	0	7	10	1	0	0	0	0	0	0	0	0	3	7	0	1	0
88	88	52	1	3	3	2	0	1	40	12	0	0	10	0	4	0	0	0	3	2	0
1	72	1	5	5	0	4	3	3	0	6	0	0	0	0	13	0	0	0	0	2	1
35	1	6	0	0	1	0	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0
18	0	2	0	3	47	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0
0	1	1	0	0	1	3	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	3	2	1	12	0	1	0	4	0	4	0	1	0	5	2	0	1	0	0
1	0	1	6	0	0	11	0	5	0	1	1	1	0	0	0	15	5	0	1	0	0
0	0	2	4	1	0	5	0	5	0	4	0	9	0	0	0	0	1	2	0	0	0
9	0	1	0	0	1	1	0	2	0	0	8	0	0	4	0	0	0	0	0	0	1
0	0	4	1	0	1	0	18	1	0	0	1	0	0	1	0	0	0	0	0	0	1
1	1	2	0	0	0	1	1	14	0	0	1	0	0	18	0	0	0	1	0	0	0
8	0	1	0	0	2	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
43	15	80	2	8	6	0	0	0	10	2	0	0	15	0	1	0	0	0	3	1	0
0	0	3	0	0	2	0	0	0	0	0	3	0	2	1	0	0	0	0	0	1	0
7	0	0	0	0	11	0	0	0	0	2	0	0	0	3	0	0	0	0	0	0	0
5	0	0	0	0	6	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
224	191	181	130	107	92	82	74	70	58	43	37	31	31	30	28	24	23	15	9	8	8

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Genetic relationships among subpopulations of the American lobster (*Homarus americanus*) as revealed by random amplified polymorphic DNA[1]

Gareth C. Harding, Ellen L. Kenchington, Carolyn J. Bird, Douglas S. Pezzack, and Denise C. Landry

Abstract: Random amplified polymorphic DNA (RAPD) profiles were used in a preliminary investigation of the genetic relationships among American lobsters (*Homarus americanus*) from the ecologically disparate and geographically separate regions of the southern Gulf of St. Lawrence, a bay off southwestern Nova Scotia, and a deep-sea canyon off Georges Bank. Phenotypic analyses of the RAPD bands showed no significant difference between samples caught at these three geographic locations. Lobsters from the Gulf of Maine, collected inshore from Lobster Bay, Nova Scotia, and offshore from Georges Bank, were genetically the most similar ($D = 0.002$), whereas Gulf of St. Lawrence lobsters were about three times as genetically distant from these two subpopulations ($D = 0.005$–0.006). However, F_{ST} values at each RAPD band ranged from <0.000 to 0.073, indicating that lobsters at these three locations are not genetically isolated. The number of migrants needed to account for this observed level of genetic differentiation could be as few as five animals in each generation. The present findings should not have been surprising given the enormous potential for larval dispersal, the wide ranging movements of adult lobsters within each region, and the level of anthropogenic interference through both displacement of larvae and adults over the past century in the name of conservation, particularly adults released into the Gulf of Maine.

Résumé : Les profils RAPD (ADN polymorphique amplifié aléatoirement) ont été utilisés dans une étude préliminaire des relations génétiques entre les homards (*Homarus americanus*) provenant de trois régions écologiquement disparates et géographiquement éloignées les unes des autres : sud du golfe du Saint-Laurent, une baie située dans le sud-ouest de la Nouvelle-Écosse et un canyon profond au large du banc de Georges. Les analyses phénotypiques des bandes RAPD n'ont pas montré de différences substantielles entre les échantillons capturés dans ces trois régions géographiques. Les homards du golfe du Maine, capturés à proximité de la côte dans la baie Lobster, Nouvelle-Écosse, et au large du banc de Georges étaient génétiquement les plus proches ($D = 0{,}002$), tandis que les homards du golfe du Saint-Laurent présentaient une distance génétique par rapport à ces deux sous-populations environ trois fois plus grandes ($D = 0{,}005$–$0{,}006$). Cependant, les valeurs F_{ST} de chacune des bandes RAPD ont varié de <0,000 à 0,073, ce qui indique que les homards vivant dans ces trois emplacements ne sont pas isolés génétiquement. Le nombre de migrants nécessaire pour expliquer ce degré observé de différenciation génétique pourrait n'être que de cinq animaux par génération. Ces constatations ne devraient pas être étonnantes étant donné l'énorme potentiel de dispersion des larves, les grandes distances franchies par les homards adultes dans chacune des régions et le degré d'intervention humaine par déplacement à la fois des larves et des adultes au cours du dernier siècle au nom de la conservation, et particulièrement des adultes qui ont été libérés dans le golfe du Maine.
[Traduit par la Rédaction]

Introduction

The most effective basis for managing a single-species fishery is to know intimately its stock structure along with the distinctive ecological characteristics of each stock. The American lobster (*Homarus americanus*) is widely distributed in both deep waters along the continental shelf break from South Carolina to The Gully, just north of Sable Island, Nova Scotia, and in coastal waters from New Jersey to the northern tip of Newfoundland (Herrick 1911). An unresolved issue is whether the generally larger, more fecund lobsters located in offshore waters represent a separate stock or stocks from the lobsters caught by the traditional inshore fishery, roughly corresponding to waters less than 20 m depth (Rogers et al. 1968; Stasko 1978; Fogarty et al. 1980; Harding et al. 1983). Controversy exists about whether certain offshore regions, such as Browns and Georges Banks off southwestern Nova Scotia, provide an important source of larvae for inshore recruitment where onshore currents prevail (Harding and Trites 1988, 1989; Pezzack 1989; Pezzack et al. 1992; Katz et al. 1994). Increased fishing effort in the offshore could be detrimental to some inshore fisheries if this were the case. The inshore lobster fishery has been subjected historically to high exploitation rates in most areas of Canada, including the southern Gulf of

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G.C. Harding.[2] Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.
E.L. Kenchington and D.S. Pezzack. Department of Fisheries and Oceans, Halifax Fisheries Research Laboratory, P.O. Box 550, Halifax, NS B3J 2S7, Canada.
C.J. Bird and D.C. Landry. National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, NS B3H 3Z1, Canada.

[1] NRCC No. 39760
[2] Author to whom all correspondence should be addressed.
e-mail: gareth.harding@maritimes.dfo.ca

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St. Lawrence and waters bordering on the Gulf of Maine, where most females are being harvested before they have a chance to reproduce (Robinson 1979; Campbell and Robinson 1983; Miller et al. 1987; Campbell 1992; Pezzack and Macquire 1995).

It is, therefore, important for conservation purposes to know which subpopulations act as reproductive units. This classification may or may not have a genetic basis. A number of investigators have considered seasonal migrations of mature lobsters, larval drift, and parasitological, morphological, physiological, and biochemical traits to identify and distinguish among lobster subpopulations or stocks (Campbell and Mohn 1982; Pezzack and Pringle 1986; Harding et al. 1993). Even lobster landing patterns from statistical reporting regions have been used as evidence for the existence of discrete stocks (Campbell and Mohn 1983; Harding et al. 1983). The cause of the observed landing patterns would be the integrated result of all interactions of population parameters with environmental variables. In the present investigation we extend this search by looking for genetic differences, using the random amplified polymorphic DNA profile technique (RAPD; Williams et al. 1990; Welsh and McClelland 1990), among subpopulations in three ecologically disparate and geographically separated locations: Arisaig in the southern Gulf of St. Lawrence, Lobster Bay in southwestern Nova Scotia, and offshore on Georges Bank. Previous publications concerned with lobster genetics (Barlow and Ridgway 1971; Tracey et al. 1975; Odense and Annand 1978; Burton and Feldman 1982; Burton 1983; Shaklee 1983; Kornfield and Moran 1989) have not directly examined nuclear DNA. However, with the availability of new molecular techniques, it is now possible to use DNA characteristics as population markers. Thus, this work reports the first attempt to obtain information on the variation of the lobster nuclear genome.

The use of RAPD markers is based on the polymerase chain reaction (Saiki et al. 1988). Short (approximately 10-base) oligonucleotide primers of arbitrary sequence are used to amplify anonymous regions of genomic DNA. The amplification products are then analyzed by electrophoresis on agarose gels and detected by staining with ethidium bromide. Variations in amplification products within a species, and the speed and relatively low cost of the technique (compared with other DNA markers), allows its use in population and other genetic studies (e.g., Allegrucci et al. 1995; Garcia and Benzie 1995; Tyler-Walters and Hawkins 1995). However, strict protocol must be followed to achieve reproducibility and visibility of the amplification products (Büscher et al. 1993). This paper represents a preliminary application of the RAPD technique to lobster DNA. We identify a number of RAPD primers that show polymorphisms in the lobster and test their frequency distribution between three subpopulations.

Methods

Sampling

The lobsters used for our population comparisons were obtained from three locations: Northumberland Strait (Arisaig, Nova Scotia, ~5–15 m depth), inshore southwestern Nova Scotia (Lobster Bay, Nova Scotia, ~10–20 m), and offshore on Georges Bank (Corsair Canyon, ~200–250 m) (Fig. 1). Sample sizes at these locations were 24, 36, and 48 lobsters, respectively, although some of the RAPD amplifications were unsuccessful and information was not obtained on every animal for each primer. Immature lobsters were chosen for this study so that the individuals sampled were more likely to be resident to the area and less likely to be immigrants or seasonally migrating animals. Lobsters from Northumberland Strait averaged 72 mm (range 66–79 mm) carapace length (CL); southwestern Nova Scotia lobsters, 75 mm (60–80 mm) CL; and Georges Bank lobsters, 74 mm (52–80 mm) CL. Lobsters were sexed, measured, and maintained in flowing sea-water tanks until required for tissue analyses. The sex ratio of the lobsters analyzed was close to unity (1.08 male : 1.00 females). Muscle tissue samples used for DNA analysis were dissected from a claw rather than the tail to avoid any contamination with foreign material in the gut.

DNA processing

DNA was extracted from both fresh, frozen (–85°C), and ethanol-preserved samples (50, 75, and 100%) taken from the same animal to determine the effect of tissue preservation on the RAPD profiles. While fresh tissue could be obtained for this study, future studies might require tissue storage prior to extraction. DNA was extracted from muscle tissue and individual claws following the protocol of Patwary et al. (1994), except that more tissue was used in the initial lysate: 1 g of tissue was ground in liquid nitrogen and immediately mixed with 3.75 mL of lysis buffer (cf. Patwary et al. 1994).

RAPD primers used in this study were obtained from Dr. John E. Carlson (Biotechnology Laboratory, University of British Columbia, Vancouver, B.C.). Forty-two primers, which had been found to be positive (producing amplification products) with sea scallop DNA (Patwary et al. 1994, Table 2 in part), were screened against one DNA sample from a single lobster from Georges Bank. The effect of tissue preservation on the RAPD profiles was assessed with 13 primers. DNA template (10 ng) was amplified using the reaction mixture described in Patwary et al. (1994). The amplification was performed in a Gene Amp PCR System 9600 (Perkin Elmer Cetus) thermocycler programmed for 40 cycles: four initial cycles of 3 min each at 94, 36, and 72°C; followed by 35 cycles of 30 s at 94°C, 1 min at 36°C, and 2 min at 72°C; and a final amplification using 1 cycle of 30 s at 94°C, 1 min at 36°C, and 10 min at 72°C. The reaction products were separated by electrophoresis at 3–4 V/cm in 1.4% agarose–TBE (Tris – borate – EDTA) gels and stained by mixing ethidium bromide with TBE in the electrophoresis running buffer to a final concentration of 0.2 μg/mL.

Of the 42 primers screened, 30 were positive with lobster DNA template (see Table 1). These 30 primers were then tested on a subset ($n = 9$) from each location to assess polymorphism. The eight most polymorphic primers were used to compare the DNA of the entire set of population samples. The resolution obtained with four of them was not consistent across gels; thus, the data from these primers were not considered further (* Table 1). UBC primers 67, 145, 153, and 205 were scored successfully for differentiation by location for this study (Table 1).

Data collection and analyses

Two data matrices were constructed based on the presence or absence of amplification products appearing as bands in the gels. In the first database, individuals were scored for the presence or absence of all 14 bands produced by UBC primer 145 (UBC 145) (Fig. 2). In the second database, the presence or absence of a clear polymorphic band from selected primers (Table 1) was recorded for each individual. Seven such bands produced by primers UBC 145 (one band; Fig. 2), UBC 205 (one band), UBC 153 (four bands; Fig. 3), and UBC 67 (one band), were scored. The data were analyzed by phenotype using chi-square analyses of bivariate tables, with variables being both individual bands and the band patterns, by location, geographic region, or sex. A pattern is defined as the sequence of either presence or absence of possible bands per individual; for example, one pattern of seven bands might be "1,1,1,0,0,0,1." The frequency of this pattern in

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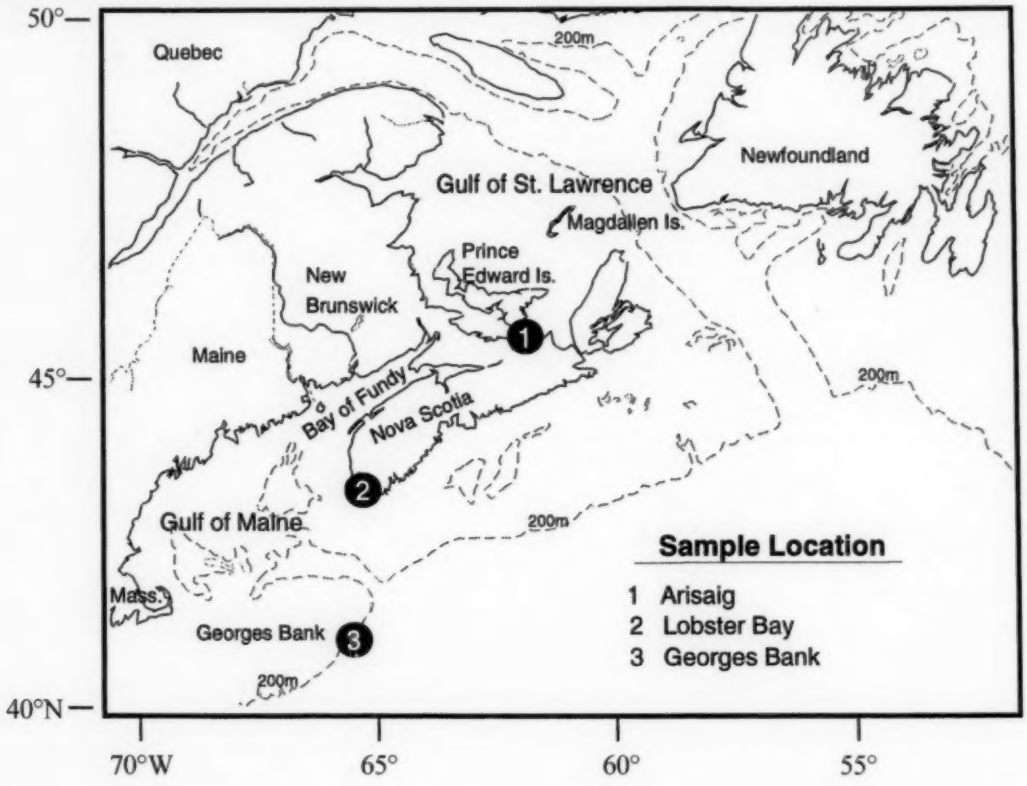
Fig. 1. Chart showing lobster collection locations in the Gulf of St. Lawrence and the Gulf of Maine.



the population becomes the data. The sequential Bonferroni procedure was employed to control for type-1 errors (Rice 1989).

The two databases were combined and the similarity between individuals calculated using a simple matching similarity measure. This is the ratio of the number of matches to the total number of characteristics. The similarity matrix was clustered using an unweighted pair-group method using arithmetic averaging (UPGMA) clustering algorithm of Sneath and Sokal (1973).

Nei's genetic distance and differentiation between locations were calculated taking into account the dominance of the RAPD markers; that is, the inability to distinguish a positive homozygote from the null allele. The analyses were restricted to those bands whose observed frequency (see Table 2) was less than $1 - (3/N)$ (Lynch and Milligan 1994). Both databases were combined for this analysis, producing data on eight bands. Genetic differentiation among the locations sampled is described by the F_{ST} statistic using the frequencies of the null allele (Wright 1978). This statistic describes the proportion of the genetic variation in the samples that is attributable to population differentiation. The effective number of migrants per generation between the locations (Wright 1943) was calculated using the island model (Wright 1978) of population structure.

Results

Pretreatment of tissue samples was found to affect the amplification. After preservation in 50% ethanol the DNA appeared to be degraded, and only the most prominent bands could be readily resolved. Neither 100% ethanol nor ultrafreezing pretreatments affected the number or resolution of the RAPD amplification products when compared with the fresh tissue control and each other. Preservation with 75% ethanol yielded results intermediate in quality between those obtained with 50 and 100% pretreatments. Thus, although we used only fresh tissue for maximum uniformity here, it would have been possible to stockpile samples for analysis in future studies.

Of the eight positive primers used to generate lobster RAPD profiles (Table 1), seven revealed clear polymorphisms whereas primer UBC 137 appeared invariant. Even when polymorphisms were present, there was a high degree of uniformity among individuals (Figs. 2 and 3). For example, 6 of the 14 bands produced by primer UBC 145 were invariant, and variation in the other bands often was restricted to a very few individuals (Fig. 2). These reactions were repeatable, and the polymorphisms were found to be stable and characteristic of each individual animal. Allele frequencies of the selected bands are given for each location sampled in Table 2.

Chi-square analysis of the RAPD bands produced by primer UBC 145 showed significant ($P = 0.03$) differences in band 10 among locations (Fig. 2, Table 2). However, this band was very difficult to score because of its proximity to band 11, rendering this result equivocal. Bands 10 and 5 were also significantly different between regions (i.e., Gulf of St. Lawrence vs. Gulf of Maine). Sequential Bonferroni testing rejected the significance of these values. Analysis by pattern showed no significant differences among locations; however, when they were grouped by region, two patterns were significantly different (Table 3; $P > 0.05$). Neither of these was statistically significant when calculated using the

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Table 1. A list of RAPD primers found to be positive with lobster DNA template in this study.

UBC primer No.	Nucleotide sequence 5′ → 3′
67	GAGGGCGAGC†
71	GAGGGCGAGG
77	GAGCACCAGG
81	GAGCACGGGG*
96	GGCGGCATGG
101	GCGGCTGGAG
102	GGTGGGGACT
104	GGGCAATGAT
105	CTCGGGTGGG*
106	CGTCTGCCCG
116	TACGATGACG
134	AACACACGAG
137	GGTCTCTCCC*
145	TGTCGGTTGC†
146	ATGTGTTGCG
149	AGCAGCGTGG*
150	GGAGGCTCTG
153	GAGTCACGAG†
174	AACGGGCAGC
181	ATGACGACGG
190	AGAATCCGCC
205	CGGTTTGGAA†
208	ACGGCCGACC
210	GCACCGAGAG
211	GAAGCGCGAT
241	GCCCGACGCG
264	TCCACCGAGC
266	CCACTCACCG
287	CGAACGGCGG
290	CCGCGAGCAC

*The resolution of these primers was not consistent across gels; therefore, they were not used to document variation among subpopulations.

†Primers scored for differentiation by location and used to evaluate variation among subpopulations.

Bonferroni procedure. Nine patterns were observed in the database, with five being unique to a single individual and three being shared by two lobsters. The two dominant patterns (58% of the lobsters) were found in 32 and 18 individuals.

The second database, produced by assembling a matrix of the presence–absence of polymorphic bands from different primers, did not show any significant differences in individual bands among lobsters from the three locations. However, one band scored on primer UBC 153-3 (band 153-3; Fig. 3) was found to be significantly ($P < 0.001$) different between the sexes. It was present in 97.9% of the females studied and in 65.4% of the males. Analysis by pattern showed significant differences among locations and between regions in one multiprimer pattern (Table 3); however, this frequency difference was not supported by Bonferroni testing. Of the 128 possible patterns, only 35 were found. Nineteen of these were unique to one individual and a further nine occurred in only two animals. The most common pattern was shared by eight individuals.

UPGMA cluster analysis did not reveal any interpretable structure in the data set. The dendogram consisted largely of individuals from different locations chaining on to one another.

The genetic distances of lobsters between sampling locations are small, indicating that these locations are not genetically isolated. Individuals from Lobster Bay and offshore on Georges Bank were the most similar ($D = 0.002$), whereas the Gulf of St. Lawrence lobsters were about three times as genetically distant from these ($D = 0.005$ with Lobster Bay and $D = 0.006$ with offshore Georges Bank). F_{ST} values at each band range from <0.000 to 0.073 (Table 4). F_{ST} values of 0.05–0.15 indicate moderate differentiation (Hartl 1980). The 145-10 band has some values within this range, but as previously stated, scoring of this band was subject to error owing to its proximity to the 145-11 band. Nevertheless, differentiation at the lower levels observed in the present study is by no means negligible (Wright 1978). The effective number of migrants per generation that would account for the level of differentiation observed between these locations could be as few as five animals (Table 4).

Discussion

Phenotypic analyses of the RAPD bands show slight differences among the lobsters sampled and that the little genetic structure observed appears to be related to the geographic region at which the lobsters were collected. The genetic distances calculated among the three locations are small, which indicates that these populations are not genetically isolated. The genetic distance is greatest between lobsters from the Gulf of St. Lawrence and the Gulf of Maine. The genetic distance calculated between the inshore and offshore lobsters in the Gulf of Maine is small ($D = 0.002$) and much less than that found between collections of scallops from similar areas ($D = 0.005$; E.L. Konchington, unpublished data). These results are perhaps not surprising given the more-or-less continuous distribution and "spawning" of the lobster in this area and the enormous potential for dispersal of the planktonic larval stages (Rogers et al. 1968; Harding and Trites 1988; Katz et al. 1994). However, this level of genetic homogeneity can be maintained with relatively few exchanges among the areas. Theoretically, one immigrant per generation should be enough to ensure common alleles but not sufficient to maintain identical allelic frequencies between subpopulations (Bohlmeyer and Gold 1991). While it is difficult to quantify precisely the level of exchange required to produce the values of F_{ST} reported in this study, especially as F_{ST} is low (Wright 1978), we estimate that as few as five migrants per generation are needed to account for the level of differentiation observed among our sampling locations. Overfishing of one of these locations would not significantly reduce the overall level of genetic variation in the species. However, given this restricted level of migration, the populations may in fact reflect separate units for management purposes.

A number of approaches have been tried in the past to separate the lobster population into stocks or subpopulations for a more meaningful and efficient management of the fishery (see Campbell and Mohn 1982; Pezzack and Pringle 1986; Harding et al. 1993). Morphological studies of adult lobsters in both Canadian and American waters have met with some success in distinguishing stocks by using multivariate techniques (Saila and Flowers 1969; Campbell and Mohn 1982). Campbell and Mohn (1982) were able to separate lobsters in the southern Gulf of St. Lawrence around to Cape Breton from

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Fig. 2. RAPD bands obtained with primer UBC 145, partial sample. Lanes 1, 29, and 42 (λ) are size markers (kb; at left) generated by digestion of lambda bacteriophage DNA with *Sty*1. Other lanes represent individual lobsters from Northumberland Strait (lanes 2–7), Lobster Bay (lanes 8–16), and Georges Bank (lanes 17–28, 30–41). Arrowheads indicate the polymorphic band 145-4 used in comparing populations with this primer; a product of about 1.5 kb present here in lanes 5, 7, 16, 33, 34, and 36.

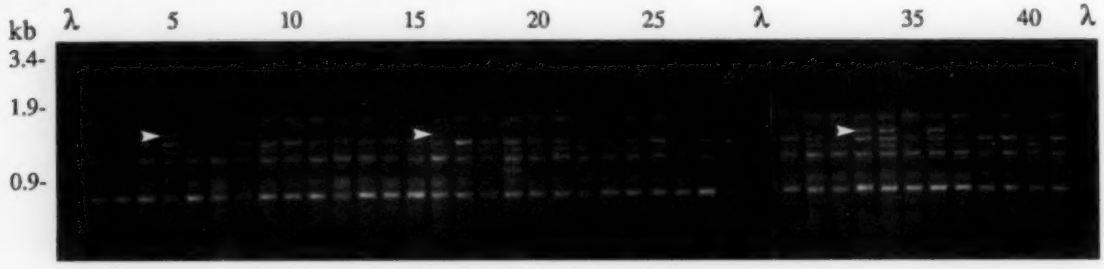
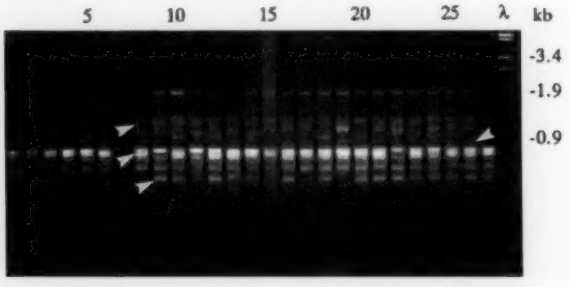


Fig. 3. RAPD bands obtained with primer UBC 153, partial sample. The rightmost lane (λ) contains size markers (kb; at right) generated by digestion of lambda bacteriophage DNA with *Sty*1. Other lanes represent individual lobsters from Northumberland Strait (lanes 1–6), Lobster Bay (lanes 7 (blank) – 15), and Georges Bank (lanes 16–27). Arrowheads indicate the four polymorphic bands scored for this primer, from top to bottom: band 153-3, 1.1 kb (which differed between the sexes); band 153-2, 0.8 kb; band 153-1, 0.7 kb; and band 153-4, 0.6 kb.



those further south along the Nova Scotian mainland, Bay of Fundy, and offshore banks. However, environmentally induced physiological differences between areas may be largely responsible for these observed morphometric differences because the onset of maturity is strongly influenced by geographical region, sea temperature, sex, and possibly other variables (Templeman 1936, 1944). In a previous study we attempted to avoid this complication between size of lobster, sexual dimorphism, and environment by studying the morphometrics of the first larval stage at various locations around the Maritimes (Harding et al. 1993). Lobster larvae were separated, by stepwise discriminant function analysis on the basis of five morphological characters, into three geographic groups: the southern Gulf of St. Lawrence and its outflow around Cape Breton Island; the Atlantic inshore region of Nova Scotia from Jeddore Harbour, Halifax County, to Lobster Bay, Yarmouth County; and the offshore banks in the Gulf of Maine. Once the effect of environmental temperature on larval size was removed, the differences between Georges and Browns banks and inshore mainland Nova Scotia disappeared. The remaining difference, now based on three morphological characters, occurred between lobsters in the southern Gulf of St. Lawrence plus its immediate outflow (Sidney Bight, Nova Scotia) and the region further south toward the Gulf of Maine and offshore banks. These three morphological differences may be due to the effect of other environmental factors or partial genetic isolation. External morphological characters of the lobster are highly dependent on environmental conditions and, therefore, are not a definitive indication of any genetic differences. In both the previous (Harding et al. 1993) and the

Table 2. Allele frequencies of band presence or absence obtained by selected RAPD primers and used for analyzing and evaluating lobsters collected from the Gulf of St. Lawrence (Arisaig, N.S., location) and the Gulf of Maine regions (Lobster Bay, Nova Scotia, and Georges Bank locations).

UBC primer	Gulf of St. Lawrence	Gulf of Maine	
No. (-band No.)	Arisaig	Lobster Bay	Georges Bank
Primer 145 bands			
145-1	1.000 (19)	1.000 (29)	1.000 (44)
145-2	1.000 (19)	1.000 (29)	0.977 (44)
145-3	1.000 (19)	0.966 (29)	0.977 (44)
145-4†	0.211 (19)	0.172 (29)	0.250 (44)
145-5	0.947 (19)	1.000 (29)	1.000 (44)
145-6	1.000 (19)	1.000 (29)	1.000 (44)
145-7	1.000 (19)	1.000 (29)	1.000 (44)
145-8	1.000 (19)	1.000 (29)	1.000 (44)
145-9	0.895 (19)	0.793 (29)	0.705 (44)
145-10*†	0.158 (19)	0.345 (29)	0.500 (44)
145-11	1.000 (19)	1.000 (29)	1.000 (44)
145-12	1.000 (19)	0.966 (29)	1.000 (44)
145-13	0.105 (19)	0.000 (29)	0.045 (44)
145-14	1.000 (19)	1.000 (29)	1.000 (44)
Prominent bands			
145-4	0.211 (19)	0.172 (29)	0.250 (44)
205	0.227 (22)	0.118 (34)	0.229 (48)
153-1	0.833 (24)	0.882 (34)	0.854 (48)
153-2	0.250 (24)	0.424 (33)	0.396 (48)
153-3	0.826 (23)	0.742 (31)	0.833 (48)
153-4	0.682 (22)	0.455 (33)	0.553 (47)
67	0.522 (23)	0.486 (35)	0.404 (47)

Note: Sample size is given in parentheses.

*Significant difference ($P < 0.05$) between locations in the chi-square analysis.

†Significant difference ($P < 0.05$) between regions in the chi-square analysis.

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Table 3. Observed and expected values of RAPD patterns showing significant ($P < 0.05$) differences between locations and (or) regions: Lobster Bay, Nova Scotia, and offshore on Georges Bank are located within the Gulf of Maine region and Arisaig within the Gulf of St. Lawrence region.

RAPD pattern	Groups	Observed (Expected)	χ^2	P
Primer 145 pattern				
1,1,1,1,0,1,1,1,1,0,1,1,0,1	Arisaig	1 (0.2)		
	Lobster Bay + Georges Bank	0 (0.8)	3.88	0.048
1,1,1,0,1,1,1,1,1,0,1,1,1,1	Arisaig	2 (0.6)		
	Lobster Bay + Georges Bank	1 (2.4)	4.00	0.045
Multiprimer pattern				
1,0,1,1,1,0,1	Arisaig	2 (0.4)		
	Lobster Bay	0 (0.6)		
	Georges Bank	0 (1.0)	7.20	0.027
1,0,1,1,1,0,1	Arisaig	2 (0.4)		
	Lobster Bay + Georges Bank	0 (1.6)	7.20	0.007

Table 4. Genetic differentiation between lobsters collected from Arisaig in the Gulf of St. Lawrence (AR), Lobster Bay, Nova Scotia (LB), and Corsair Canyon on Georges Bank (GB) in the Gulf of Maine, as described by the F_{ST} statistic.

UBC Primer No. (-band)	F_{ST}			
	AR–LB–GB	LB–GB	LB–AR	GB–AR
145-4	0.003 (77)	0.005 (51)	0.001 (193)	0.001 (218)
205	0.009 (28)	0.011 (22)	0.011 (23)	0.000 (82 322)
153-2	0.014 (18)	0.000 (536)	0.019 (13)	0.013 (18)
153-3	0.008 (30)	0.010 (25)	0.008 (30)	0.000 (3 373)
153-4	0.023 (11)	0.006 (43)	0.033 (7)	0.012 (21)
67	0.006 (44)	0.004 (63)	0.001 (319)	0.008 (30)
145-10	0.048 (5)	0.014 (17)	0.025 (10)	0.073 (3)
145-13	0.020 (13)	0.012 (22)	0.028 (9)	0.007 (37)

Note: The number of migrants which would account for the level of differentiation observed (F_{ST}) is given in parentheses, assuming the island model of population structure. Seven decimal places were used in calculations, and the results were rounded to nearest animal.

present study, however, the Gulf of St. Lawrence lobsters appear distinctive.

Enzyme electrophoresis, restriction enzyme analysis of mitochondrial DNA, and amino acid sequencing are relatively independent of environmentally induced changes to the phenotype and would provide evidence for or against any geographic segregation and reduced gene flow in the lobster population. Several studies of lobster genetics have been conducted over the past 20 years. The majority of these were based on enzyme electrophoresis. All of the enzyme electrophoretic studies found a high degree of homogeneity. Barlow and Ridgway (1971) first used starch-gel electrophoresis on lobsters to examine variation in the esterase isozymes in a search for genetic differences between inshore and offshore populations in the Gulf of Maine. No difference was observed in the phenotypic frequencies of lobsters captured near Boothbay Harbor, Maine, and either the Hudson or Lydonia Canyons off Georges Bank. Odense and Annand (1978) compared 12 proteins or enzymes electrophoretically from tissue extracts of inshore and offshore lobsters ($N = 200$) collected off southwestern Nova Scotia and found no evidence for a genetic basis of distinguishing individuals from the two areas. Tracey et al. (1975) examined 18 enzymatically different proteins, using electrophoretic techniques, in 300 lobsters collected from the Gulf of St. Lawrence, Bay of Fundy, coasts of Maine and Massachusetts, and three locations near offshore canyons. They found generally low levels of genetic variability at the 44 loci examined, with almost all this variability confined to 8 loci. All but one of their allelic frequencies were geographically uniform. Differentiation between some areas was found at the malic enzyme loci, but weak activity of this enzyme in lobsters from the Bay of Fundy region and the coast of Maine did not allow the determination of allelic frequencies there. Shaklee (1983) had reservations about the results and interpretation given by Tracey et al. (1975), because only 42% of their lobsters were screened successfully for the malic enzyme. Heterozygotes could have made up a disproportionate number of unscorable individuals, because the activity is distributed among two or more isozymes. There is also some question about the true identity of their malic enzyme, because previous authors using other organisms had found a tetrameric protein structure rather than the monomeric structure reported by Tracey et al. (1975). Burton and Feldman (1982), Burton (1983), and Shaklee (1983) have re-examined the results of Tracey et al. (1975) and showed that some significant genetic differentiation may exist between some sampling sites at other loci. However, Shaklee (1983) concluded that further research is warranted because the results are limited by weak enzyme activity, small and uneven sample sizes, and other uncertainties.

There is a problem of cryptic heterogeneity in all electrophoretic studies whereby additional allozymes may be hidden but can be detected by their differential stability to heat,

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movement on different pore-sized gels, and pH of the medium (Johnson 1977). In other words, the observation of differential mobility on a single gel, under set conditions, is evidence for establishing a protein structural difference between individuals, whereas the converse is not sufficient to establish identity.

More recently, Kornfield and Moran (1989) attempted to characterize lobsters taken from the Gulf of St. Lawrence, the Gulf of Maine, and the Atlantic slope off Delaware with mitochondrial DNA restriction enzyme digestion patterns. Mitochondrial DNA in general acquires nucleotide substitutions at a higher rate than nuclear DNA (Brown et al. 1979), rendering it useful for population genetic studies (e.g., Palumbi and Benzie 1991). They revealed numerous distinctive genotypes, several of which were common throughout. Cladistic and phenetic analysis of relationships among genotypes indicated the presence of two major lineages, which suggests that the lobster population may have had a fragmented geographical distribution in the past. The degree of differentiation found indicated that these lineages originated before the flooding and repopulation of the Gulf of St. Lawrence and Gulf of Maine, following the retreat of the glaciers, which started 14 000 years BP. The relative frequencies of these two lineages did not differ significantly among the localities sampled, although there was an indication of a divergence in genotype frequency between the Gulf of St. Lawrence and Gulf of Maine.

This slight genetic distinction found between lobsters from the southern Gulf of St. Lawrence and Gulf of Maine in the present study and in previous morphological, enzymatic, and mitochondrial DNA studies might have evolved in response to the predominantly "one-way" drift of larvae from the Gulf of St. Lawrence, forming a partial hydrographic barrier to gene flow. In other words, natural selection may have been operating on the lobster over the past 5000 years for adaptation to the warmer summer environment of the southern Gulf of St. Lawrence. The southern Gulf of St. Lawrence has a relict warm-water fauna that has been separated from its counterpart south of Cape Cod since the Labrador Current inundated the submerging Grand Banks and thereby initiated a general climatic cooling trend on the east coast (see Bousfield and Thomas 1975).

Another factor that could influence the genetic structure of the lobster population geographically is the movement or migrations of adults, which can be extensive and cover considerable distances in the Gulf of Maine region and its offshore banks (Uzmann et al. 1977; Krouse 1980; Campbell et al. 1984; Anonymous 1985; Campbell and Stasko 1986; Campbell 1989*a*). However, there is also evidence that some females and males undergo seasonal return migrations of up to 200 km, which would place their mating activities and larval release at a specific inshore (Campbell 1986; Campbell and Stasko 1986) or offshore (Pezzack and Duggan 1986) spawning ground each summer. Tagging studies have shown a fair exchange of mature lobsters between inshore and offshore waters off southwestern Nova Scotia, with few lobsters reaching as far afield as Georges Bank (Campbell and Stasko 1985; Campbell 1989*a*, 1989*b*). However, long-range movement of adult lobsters from the outer Atlantic coast of Nova Scotia to offshore banks is extremely rare (Miller et al. 1989; R.E. Duggan, Department of Fisheries and Oceans, P.O. Box 550, Halifax, NS B3J 2S7, Canada, personal communication). There are no records of long-range movements of adult lobsters out of or into the Gulf of St. Lawrence.

Finally, an issue that has not been discussed at length to date and may well have had far-reaching effects on the genetic integrity of the lobster population is the early conservation and marketing practices of both Canada and the United States (Harding et al. 1993). Lobster hatcheries were first established in the United States at Woods Hole, Mass., in 1885 and in Canada at Bay View, N.S., in 1891 (Bowers 1900; Rodd 1917). The proliferation of hatcheries to 30 installations by the early 1900s was a conservation measure to counteract the then unenforceable law prohibiting the sale of egg-bearing ("berried") females (Rodd 1917; Carlson 1954; Wilder 1965). Eggs were removed from lobsters collected either at the canneries or the wharf and held until they hatched (Bowers 1900; Knight 1918; MacKay 1929; Carlson 1954). The object was to restock heavily fished lobster grounds with newly hatched larvae. A few Canadian and American hatcheries cultured lobster larvae through to the settling stage (stage IV) before releasing them (Knight 1918; Carlson 1954). However, the survival rate of larvae in the hatcheries was later assessed to be much lower than that achieved by returning berried females to the sea (Knight 1918, Taylor 1950). As a consequence, all Maritime installations were closed after 1917 (Knight 1919) and most of the New England hatcheries by the 1920s, with only a few hatcheries devoted to producing settling-stage lobster continuing in operation into the 1950s (Carlson 1954). One hatchery apparently remains in operation: the Massachusetts State Lobster Hatchery in Martha's Vineyard, Mass., releasing 500 000 stage IV lobsters per year (Waddy 1988).

The significance of this early conservation practice is that lobster eggs were collected along extensive tracts of coastline and returned as larvae not necessarily in the area of their origin. For example, spawn collected for the earliest hatchery in the United States at Woods Hole, Mass., came from as far away as New London, Conn., and Plymouth, Mass., in the southern Gulf of Maine (Bowers 1900). Spawn was generally collected within 50 km of the hatcheries in Canada; nevertheless, there were occasions where larvae were introduced far from their place of origin, such as larvae from the Canso, N.S., hatchery being used to repopulate Bedford Basin, Halifax Harbour, Nova Scotia (Rodd 1917).

It is difficult to evaluate this exchange of genetic material by larval introductions along the coast. Most larvae cultured in Canada were released reasonably close to their origin; however, larval "seeding" was much more widespread in the United States, at least at the turn of the century. The low survival of hatchery-reared organisms, however, may mean that larval introductions had a minor effect on the genetic composition of the lobster population along the coast.

A more serious management effect on the local genetic composition of the lobster population is the practice of releasing berried and undersized ("shorts") lobsters in foreign waters. Venning (1874) makes first reference to the fact that females and young lobsters were introduced intentionally from one region of Maine to another to replenish stocks. Knight (1919) quotes from the Massachusetts Fish and Game Commissioner's report of 1917 that 37 000 shorts and 200 berried lobsters were seized in the Boston market, mainly from Nova Scotian and New Brunswick sources, and released between Cape Ann and Provincetown, Mass. A further 1300 berried

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lobsters were purchased for the explicit purpose of seeding American waters. This practice continued up to at least 1932 when 30 281 shorts and 2910 berried lobsters, largely from Canadian sources, were released in American waters off Massachusetts (Chaisson 1932). Chaisson estimates that 22 000 such lobsters, on average, were released each year from the Boston market between 1909 and 1932. This represents a massive influx of genetic material. The practice was by no means limited to the Boston area. Templeman (1944) commented that many Gulf of St. Lawrence lobsters kept in pounds at Grand Manan, New Brunswick, were liberated locally when they became berried. Similar releases and mass escapes of lobsters are common from the large pound on Deer Island, New Brunswick (P.G. Wells, Environment Canada, 45 Alderney Drive, Dartmouth, NS B2J 2N6, personal communication). In the summer of 1987, the Grand Manan Fishermen's Association and Department of Fisheries and Oceans purchased 842 female lobsters from Newfoundland and 155 local lobsters, which had become "berried" in Grand Manan pounds, for a local tag and release conservation initiative (Robichaud 1990). Most of the Newfoundland lobsters recaptured from this field experiment were taken within 10 km of the release site (95%) over a 3-year period and did not undergo the distinctive deep-water migration documented for the local lobsters of this region (Robichaud and Campbell 1995). In the spring of 1995 an estimated 16 000 berried lobsters from the Magdellan Islands were released by the Maine Lobstermen's Association along the coast to increase larval production (J.S. Krouse, Maine Department of Marine Resources, West Boothbay Harbor, ME 04575, U.S.A., personal communication). Obviously the genetic exchange between geographic regions has been extensive, and the practice is continuing. This wholesale introduction of lobsters must have had an effect at the population level in the Gulf of Maine. Not surprisingly, it appears from the Grand Manan field experiment that introduced foreign lobsters are not as well adapted to local conditions, but it is unclear whether this introduction will dilute the local genome and make the local population less fit in the future.

In conclusion, knowledge of the local life history of the lobster indicates that adult migration and larval dispersal (gene flow) can be considerable in the Gulf of Maine. The present study indicates that lobster population genetic divergence appears to be minimal within the Gulf of Maine and not much more when comparing the Gulf of Maine with the Gulf of St. Lawrence. However, the estimated number of migrants per generation between all locations is low (less than 100) in an ecological context. The present subpopulations may represent separate management groups, or stocks, when their population dynamics are being considered, although their genetic differentiation is small. Future efforts to find genetic distinctions between various inshore and offshore lobster populations around the Gulf of Maine may be confounded by the continuing introduction of alien lobsters into the region.

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Addendum

Since the submission of this paper, a publication by Tam and Kornfield (1996) has appeared which uses microsatellite DNA markers to examine the relationship between a single population of American lobster and the European lobster (*Homarus gammarus*).

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Bioenergetics of Arctic cod (*Boreogadus saida*) at low temperatures[1]

Haakon Hop, William M. Tonn, and Harold E. Welch

Abstract: The Arctic cod (*Boreogadus saida*), a key component of the marine food web of Canadian high Arctic waters, occurs at temperatures close to or below zero. We established a complete energy budget for this Arctic fish, based on laboratory measurements of consumption (C), growth (G), respiration (R), egestion (F), and excretion (U) at about 0°C. An average ($N = 3$) energy budget for individual juvenile Arctic cod fed live Arctic zooplankton was $100C = 50G + 24R + 9SDA + 3F + 6U + 8Z$, where SDA is specific dynamic action (derived from previously published studies) and Z included activity and unaccounted energy. Eight determinations of total energy budgets for fish fed to satiation accounted for 72–104% of the energy consumed. Maximum daily rations and growth rates were dependent on fish size and the energy content of prey. Although maximum daily rations were similar for small fish fed different prey, diets of *Calanus* copepods (high energy content) resulted in faster growth rates than diets of *Themisto* amphipods or capelin (*Mallotus villosus*) fillets. Both consumption and respiration rates were low at temperatures close to 0°C. The net result was slow growth rates, although the assimilation efficiency was about 80%. Because of slow growth of individuals, the relatively high annual production of Arctic cod mainly reflects their large standing biomass.

Résumé : La morue polaire (*Boreogadus saida*), qui est un élément clé du réseau alimentaire marin dans les eaux de l'Extrême-Arctique canadien, vit à des températures proches de 0°C ou inférieures à 0°C. Nous avons dressé un bilan énergétique complet de ce poisson de l'Arctique, bilan fondé sur des mesures en laboratoire de la consommation (C), de la croissance (G), de la respiration (R), de l'éjection (F) et de l'excrétion (U) à environ 0°C. Le bilan énergétique moyen ($N = 3$) pour des morues polaires juvéniles individuelles nourries avec du zooplancton arctique vivant était : $100C = 50G + 24R + 9SDA + 3F + 6U + 8Z$, où SDA est l'activité specifique dynamique et Z comprenait l'activité et l'énergie non imputée. Huit déterminations des bilans énergétiques totaux des poissons alimentés jusqu'à satiété ont rendu compte de 72–104 % de l'énergie consommée. Les rations quotidiennes maximales et les taux de croissance dépendaient de la taille du poisson et de la teneur énergétique des proies. Bien que les rations quotidiennes maximales aient été semblables pour les poissons de petite taille alimentés avec des proies différentes, les régimes alimentaires constitués de copépodes du genre *Calanus* (haute teneur énergétique) ont donné des taux de croissance plus rapides que les régimes alimentaires constitués d'amphipodes du genre *Themisto* ou de chair de capelan (*Mallotus villosus*). Les taux de consommation et de respiration étaient plus élevés aux températures proches de 0°C. Les résultats nets étaient des taux de croissance lents, bien que l'efficacité d'assimilation ait été d'environ 80 %. Du fait de la lente croissance des individus, la production annuelle relativement haute de la morue polaire reflète principalement la forte biomasse vivante de cette espèce.
[Traduit par la Rédaction]

Introduction

The Arctic cod (*Boreogadus saida* Lepechin, 1774) is a key component in the high Arctic marine food web (Bradstreet et al. 1986; Hobson and Welch 1992; Welch et al. 1992), with annual predation by sea birds and marine mammals estimated to be in the order of 148 000 t in the Lancaster Sound region of Canada (Welch et al. 1992). Estimates of the energy flow through parts of the food chain have been made, including preliminary values for annual ingestion and growth by Arctic cod (Welch et al. 1992). There appeared to be a major discrepancy between estimates of Arctic cod abundance or production and total annual predation on cod, with the estimates of cod abundance in the Lancaster Sound region being too low to account for consumption by marine mammals and seabirds (Welch et al. 1992). However, these estimates were based on dispersed cod, and probably a substantial proportion of the biomass is aggregated in schools (Crawford and Jorgenson 1990, 1993, 1996; Welch et al. 1993). The densities and biomasses of such schools have recently been estimated (Crawford and Jorgenson 1996), and the cumulative biomass of such schools may represent the energy source needed to balance the trophic model of Welch et al. (1992). However, better estimates of energy flow through Arctic cod will aid in the assessment of its production. A step in this process is to establish daily consumption and growth rates of the Arctic cod and develop an energy budget for this species.

Field estimates of daily consumption rates are generally not very accurate, because it is impossible to measure consumption rates directly. They can be inferred from measurements of gastric evacuation rates and mean stomach fullness (e.g., Bajkov

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H. Hop[2] and W.M. Tonn. Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.
H.E. Welch. Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada.

[1] Contribution No. 25 from the Resolute Marine Laboratory, Resolute, N.W.T.

[2] Author to whom all correspondence should be addressed. Present address: Norwegian Polar Institute, Box 399, N–9001 Tromsø, Norway. e-mail: haakon.hop@tromso.npolar.no

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1935; Jobling 1982; Dwyer et al. 1987; Hop 1994), but the variability in stomach fullness of Arctic cod is high (Hop et al. 1997). In this study we used an experimental approach to feeding energetics at low temperatures. In experimental studies, daily rations can be controlled and resulting growth rates monitored, which makes it possible to calculate growth efficiencies directly.

Many studies of growth efficiencies based on controlled food intake exist for other gadid species: Atlantic cod (*Gadus morhua*) (Edwards et al. 1972; Jones and Hislop 1978; Jobling 1988), Pacific cod (*Gadus macrocephalus*) (Paul et al. 1990*a*), and walleye pollock (*Theragra chalcogramma*) (Smith et al. 1986; Paul et al. 1990*b*). However, only limited information on feeding energetics is available for Arctic cod. Jensen et al. (1991) measured growth and daily consumption rates for a group of Arctic cod from the Barents Sea that were fed peeled shrimp at –1.0°C. Little experimental work, apart from the study by Jensen et al. (1991) has been done on feeding energetics of Arctic marine fishes at Arctic temperatures, presumably because of limited laboratory facilities that can sustain fish at low temperatures (close to 0°C) for sufficient periods of time. Christiansen and George (1995) determined that oil-contaminated food pellets did not affect food intake, although it did affect growth measured at 3–6°C. Holeton (1974) determined respiration rates for different sizes of Arctic cod at a single temperature (–1.5°C), whereas Hop and Graham (1995) included different temperatures (–0.5 to 2.7°C) and the effect of acclimation and food intake. The present study differed from the previous studies on Arctic cod in that we used live Arctic zooplankton fed to individual fish, at low ambient temperatures (about 0°C), and determined a complete energy budget for Arctic cod. Prior to this study, the only other energy budget that existed for a polar marine fish is that of the Antarctic cod (*Notothenia coriiceps neglecta*) (Everson 1970; Crawford 1979).

This paper, which is based on Hop (1994), is the first to establish a complete energy budget for an Arctic marine fish, where consumption = growth + respiration + waste losses (Brett and Groves 1979). In this paper we report daily rations and growth rates of Arctic cod based on data from controlled feeding experiments on individuals and groups fed different diets and rations. Our experiments were carried out at the Resolute Bay Marine Laboratory, Department of Fisheries and Oceans, Canada, in Resolute, N.W.T. (74°42′ N, 95°50′ W), and we also report results from one set of experiments conducted at the Ocean Sciences Center, Memorial University of Newfoundland, in St. John's.

Materials and methods

We conducted the experiments over the course of 3 years, using Arctic cod from Resolute and Allen bays (about 74°N, 95°W) on the south coast of Cornwallis Island, Northwest Territories. Specimens were collected from subsamples of trap-net catches to attain a desirable size range for each experiment: 1988 range was 68–254 mm; 1989 range was 80–101 mm; and 1990 range was 108–128 mm.

Feeding experiments (1988): variable fish size, diet of capelin

Arctic cod, collected in mid-August, were kept for a few days in the Resolute Marine Laboratory, before being shipped to St. John's, Nfld. Fish were put in large, clear plastic bags that contained about 5 L of water on the bottom, with 5–10 individuals per bag, depending on fish size. The bags were filled up with oxygen prior to sealing them, and they were placed inside 90-L insulated coolers that had a layer of crushed ice on the bottom. Transit time was about 24 h, during which time the temperature had increased <2°C, and there was no mortality. At the Ocean Sciences Center fish were held in two cooled, 380-L holding tanks containing seawater, 29‰ salinity at about 0°C (variability specified in Results). The fish were acclimated to the tanks for about 1.5 months and became accustomed to feeding on chopped frozen capelin (*Mallotus villosus*) fillets within 2–3 weeks; fish were maintained on this food until the experiment started.

The experimental setup consisted of four glass aquaria (37.5 L) inside two reservoir tanks. Each glass aquarium was divided by perforated (3-mm PVC screen) walls into four compartments with various volumes of water (large, 10 L; medium, 8 L; small, 6 L) to accommodate different-sized fish. Experimental compartments were divided equally between the two reservoir tanks, and one additional compartment (18.5 L) in each reservoir tank contained a group of twelve fish. A small pump inside each reservoir tank supplied water (about 1 $L \cdot min^{-1}$) to individual compartments through a branched hose. Water overflowed back to the reservoir, through screens (1.5-mm mesh) near the upper edge of each glass aquarium. Ambient salt water supplied to each reservoir tank was 0.5–1.0 $L \cdot min^{-1}$. A screened frame covered the glass aquaria, and each reservoir tank was covered by an insulated lid. Fish were thus kept in darkness except during cleaning and feeding (3–4 $h \cdot day^{-1}$). Temperature was monitored daily during the experiment, and salinity was determined biweekly.

The experiments, which lasted 56 days (mid-October to mid-December), included four treatments: individual fish fed maximum ($n = 14$) and reduced rations ($n = 12$), and groups of fish (fork length range 150–199 mm, marked individually with combination fin clips) fed maximum rations ($n = 12$), and maximum rations during the first month followed by starvation ($n = 12$). Fish on maximum ration were fed to satiation, whereas fish on reduced ration were fed rations which were reduced by half every 2 weeks (2, 1, 0.5, 0.25% of body weight). Freshly thawed and chopped capelin fillets were weighed (to the nearest 0.01 g) prior to feeding, which occurred on alternate days, and excess food was removed from the compartments, blotted on tissue paper, and reweighed. Samples of capelin fillets (10 g) were taken every 4 days throughout the experiment; dry weight (%) and energy content of capelin fillets did not change significantly (energy content = 24.383 ± 0.205 kJ·g dry $weight^{-1}$ (mean ± SE), $n = 16$, $p = 0.540$). All fish (anesthetized in MS-222) were measured (to the nearest millimetre) and weighed (to the nearest 0.01 g) 5 days after the feeding schedule began (day 0), and then every 2 weeks until the experiment was terminated (day 56).

Faecal matter was carefully siphoned out of individual compartments daily into small (45-mm wide) hexagonal trays, and water was decanted. Faeces were dried for 24 h at 60°C, pooled for 2 days of collecting, and weighed (to the nearest 0.001 mg).

At the end of the experiment, fish were frozen and later dissected with the following tissues weighed: carcass, liver, gonads, and intestines (less stomach contents). Arctic cod ($n = 100$), collected by trawl from Resolute Bay in late July – August, and dissected in the same manner, served as standards. All tissues were dried at 60–70°C to constant weight (4–5 days).

The caloric density of dried, homogenized samples (1.0 g) of tissues and faecal matter were determined by adiabatic bomb calorimetry (Adiabatic bomb calorimeter 1241) with results expressed in joules. The procedure was standardized with benzoic acid pellets ($n = 10$) of known energy content (26 444 $J \cdot g^{-1}$), and resulting energy values were corrected for combustion of fuse wire and formation of nitric acid. In addition, total protein was determined by the Kjeldahl nitrogen procedure (Bradstreet 1965), and total lipids by methanol–chloroform extraction (Folch et al. 1957).

Feeding experiments (1989): uniform fish size, diets of *Calanus* and *Themisto*

Fish, collected during 14–20 July, were kept in flow-through holding

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tanks with seawater of about 30‰ salinity, in the Resolute Marine Laboratory for 2 weeks, during which time they became conditioned to feeding on live Arctic zooplankton. These included adult copepods *Calanus* spp., predominately *C. hyperboreus* and *C. glacialis*, and juvenile amphipods *Themisto* spp., predominately *T. libellula*; these diets are subsequently referred to as *Calanus* and *Themisto*. A uniform length group of fish that were in good condition (with no external damage and normal feeding behavior) were selected for the experiments.

The experimental tanks, designed for feeding experiments on individuals, consisted of two fibreglass tanks with individual boxes mounted inside. The tanks were 180 cm long, 45 cm wide and 30 cm deep, and 10 Plexiglas boxes (20 × 11.5 × 23 cm with walls of 1.5-mm black screen) were glued to the bottom of each tank. Before experiments started, all fish were conditioned for 1 week to feed individually in these boxes.

Water was pumped from Resolute Bay at 5–6 m depth and was filtered through a screen (0.8 mm) before entering a settling tank (200 L). A pump with ceramic prefilters and foam filters pumped water from the settling tank into each of the experimental tanks. Water flowed into each tank at one end through a perforated pipe, and an overflow pipe at the other end kept the water level at 21 cm. Sediment and particles in the settling tank and foam filters were removed daily. Temperatures in the tanks were also measured daily (see Results). Illumination was not controlled. Lights in the lab were normally off between 22:00 and 09:00, although windows close to the tanks provided dim natural lighting.

The experiments, which lasted 48 days (early August – end of September), contained five treatment groups. One experimental group ($n = 10$) was fed *Calanus* and one group ($n = 10$) *Themisto* daily. Within each group half of the fish ($n = 5$) were fed to satiation, with the other half being fed reduced rations at approximately maintenance levels, which were estimated based on respiration values for Arctic cod (Holeton 1974) and general energy budgets (Brett and Groves 1979). These rations were initially set too high, so after 24 days they were lowered from two to one prey daily. In addition, a starvation group ($n = 8$) was included. Fish were anesthetized in MS-222 and measured and weighed every 12 days, and faecal matter was collected as described previously. Dried faecal matter from each fish was pooled for each 12-day period before being weighed (0.1 mg).

Each fish in its box was fed a number of live zooplankters. Satiation was assumed when a fish refused five times to take additional individual zooplankters delivered by a pipette. Zooplankton remaining in each box after each feeding were removed and counted. We attempted to use single size-classes of *Calanus* or *Themisto* throughout the experiment, and lengths and weights of prey were periodically measured. Daily rations were determined based on numbers of prey consumed and weights (mean ± SE) of individual *Calanus* (11.00 ± 1.65 mg) and *Themisto* (20.49 ± 1.28 mg) in samples of 250–650 individuals.

Zooplankton was obtained every 3–4 days from Resolute Passage, with a large plankton net (1-m diameter, 0.9-mm mesh), towed horizontally at various depths. The plankton was sorted through screens of different mesh size into the two prey groups and kept in flow-through, aerated holding tanks (200 L). Samples of plankton were counted, weighed, and dried before caloric densities were measured by bombing, although potential changes in caloric values of zooplankton collections over time were not determined.

Arctic cod ($n = 15$) were collected by trawl from Resolute Bay on 9 August, close to the time when the feeding experiment started. These fish served as standards for caloric determinations of experimental fish. Caloric density, total lipids, and total protein of zooplankton, fish, and faecal matter were determined as previously described.

Feeding experiment (1990): uniform size group, diet of *Calanus*

This experimental setting was similar to that of the 1989 experiments, with the following differences. Fish used in experiments were larger, and they were fed only *Calanus*; both numbers and weight (0.1 mg) of daily individual rations were determined. The experiments, which lasted 36 days (mid-August to late September), contained three treatments: maximum (satiation) ration ($n = 8$), reduced (close to maintenance) ration ($n = 10$), and starvation ($n = 10$). Reduced ration levels were three *Calanus* daily. Two fish in the maximum ration treatment (initially, $n = 10$) were excluded from growth calculations, because they rarely fed during the initial period and thus lost weight.

The filter system was improved over the 1989 version; the water was additionally filtered through a 0.55-mm screen and then through a 10-cm thick filter-floss mat (replaced weekly), placed inside a 50-L tub on top of each settling tank. The additional filtering was necessary because we noted that small harpacticoid copepodites, occurring in the water supply in September, would pass through the previous filter system.

Energy budgets

Daily rations were determined as the mean number of individual zooplankters and the wet weight of zooplankton or capelin fillets consumed per day. Growth for a slow-growing fish, such as Arctic cod, was approximately linear over the experimental periods, and we therefore used linear growth rate models rather than instantaneous models (Jobling 1983). The absolute (G_1) and relative (G_2) growth rates were determined as $G_1 = (W_2 - W_1)/(t_2 - t_1)$ and $G_2 = G_1/W_1 \times 100$, where W_1 and W_2 denote wet weight (g) at time t_1 (day 0) and t_2 (last day of experiment), respectively (Busacker et al. 1990).

Parameters in the subsequent equations were based on values converted to energy equivalents. The energy budget (Brett and Groves 1979) for Arctic cod was calculated as $C = G + R + F + U + Z$, where C is consumption, G is growth, R is respiration + specific dynamic action (SDA), F is egestion (faeces), U is excretion (of ammonia), and Z is activity and unaccounted energy. Budget components (right side of equation) were expressed as percentages of consumption.

Gross growth efficiency (GGE), the capacity to convert food into flesh (Ivlev 1939; Welch 1968) was GGE = $(G/C) \times 100$. Assimilation efficiency (AE; Welch 1968) was AE = $(G + R)/C \times 100$ and net growth efficiency (NGE; Ivlev 1939; Welch 1968) was NGE = $G/(G + R) = G/A$, where A is assimilated energy.

Consumption (C) during each experimental period was determined from daily rations and energy contents of food.

Growth (G) in wet weight was converted to energy equivalents based on the dry/wet weight ratio and mean caloric content of dried Arctic cod tissues. Change in energy content of experimental fish was accounted for.

Respiration (R) was determined by measuring oxygen consumption of starved and fed Arctic cod (Hop and Graham 1995). The values used here were routine metabolic rates, determined as oxygen consumption rates for short-term acclimated (2 weeks) fish at ambient temperatures (–1.5 to 0.5°C) at the Resolute Marine Laboratory. Rates were adjusted to the mean temperature and fish size in each of our experiments, based on relationships between oxygen consumption and temperature (Hop and Graham 1995) and size (Holeton 1974). Oxycalorific coefficients (Q_{ox}) were determined based on the substrate being respired; $Q_{ox} = 13.72$ for lipids, and 13.36 for protein (Brett and Groves 1979; Brafield 1985). Starved Arctic cod likely use energy stored in the energy-rich, large liver for respiration. A lipid:protein ratio of 14.94 in the liver results in $Q_{ox} = 13.70$ J·mg^{-1} O_2. Respiration energy in fed fish was assumed to originate from the diet. The lipid/protein ratio was determined to be 0.24 for capelin, 1.62 for *Calanus*, and 0.65 for *Themisto*, which resulted in Q_{ox} of 13.43, 13.58, and 13.50 J·mg^{-1} O_2, respectively. Because fish were individually confined to boxes, they were generally not very active. We did not account for activity, but attempted to estimate its magnitude based on maintenance energy (i.e., budget where G = 0) in the 1990 experiment.

Egestion (F) was calculated from the dry weight of faecal matter collected daily from fish in experiments, and the energy content of

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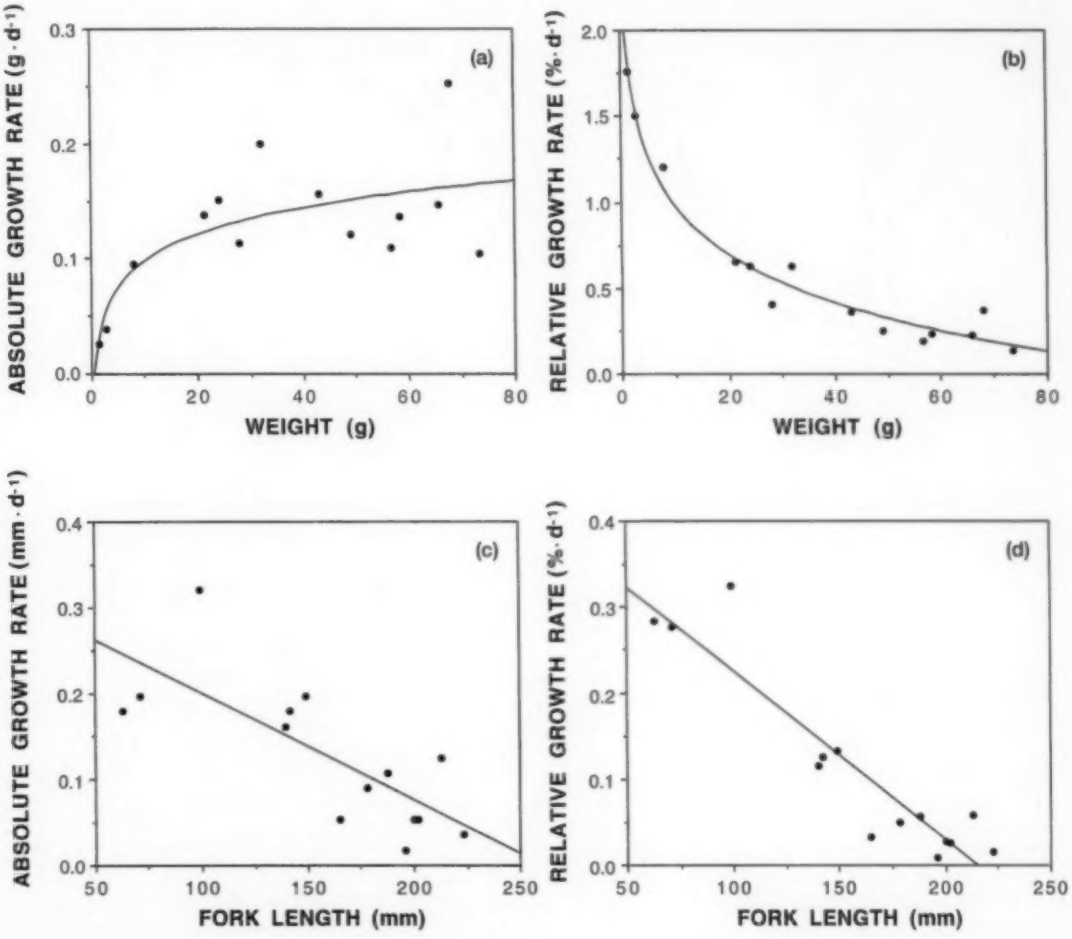
Fig. 1. Growth rates of Arctic cod (W_1, initial weight; L_1, initial length) fed satiation diets of capelin, in 1988 experiments. (*a*) Absolute growth rate (g·day^{-1}) fitted by logarithmic regression: $G_1 = 0.020\,68 + 0.033\,36 \ln(W_1)$, $r^2 = 0.512$. (*b*) Relative growth rate (%·day^{-1}) fitted by logarithmic regression: $G_2 = G_1/W_1 \times 100 = 1.9248 - 0.4101\ln(W_1)$, $r^2 = 0.971$. (*c*) Absolute growth rate (mm·day^{-1}) fitted by linear regression: $G_1 = 0.324\,15 - 0.001\,24\,L_1$, $r^2 = 0.598$. (*d*) Relative growth rate (%·day^{-1}) fitted by linear regression: $G_2 = G_1/L_1 \times 100 = 0.421\,14 - 0.001\,96\,L_1$, $r^2 = 0.865$.



pooled dried feacal matter from groups of Arctic cod fed the same food items (to get sufficient quantity for caloric determinations).

Excretion (U) was determined from ammonia in water samples, taken after 2–3 h, from closed chambers containing juvenile Arctic cod that were starved or fed krill (*Euphausia pacifica*), having a mean energy content 4.897 kJ·g wet weight^{-1}. Samples were kept in the dark on ice until analyzed. Total ammonia ($NH_3 + NH_4+$) was analyzed in diluted (1:10) samples on a Technicon autoanalyzer according to the procedure of Stainton et al. (1977). We used 20.5 J·mg^{-1} excreted ammonia as the conversion value (Brafield 1985).

Results

Temperature

Temperatures in the three experiments were generally within ±1.0°C of zero. In the 1988 experiment the daily temperature was relatively constant, 0.10 ± 0.02°C (mean ± SE). In 1989 and 1990 temperatures fluctuated more and dropped slightly over the course of the experiments. This reflected ambient conditions, as the temperature in the tanks was generally within 0.2°C of ambient water temperature. Daily temperatures during experiments were 0.01 ± 0.10°C in 1989 and –0.18 ± 0.11°C in 1990.

Growth rates

When fish were fed at maximum rations of capelin, both absolute (G_1) and relative (G_2) growth rates depended on the initial weight or length of fish. Lighter fish had lower absolute rates of growth than heavier fish, with rates stabilizing for fish >20 g (Fig. 1*a*), although relative rates of growth had the opposite relationship (Fig. 1*b*). On the other hand, shorter fish had higher absolute and relative growth rates than longer fish (Figs. 1*c* and 1*d*). When individual fish were given maximum rations of capelin, the heavier fish (>20 g) grew 147–150 mg·day^{-1}, whereas lighter fish (<10 g) grew only 53 mg·day^{-1} (Table 1, Fig. 2*a*).

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Table 1. Absolute and relative growth rates, daily rations, and faecal matter of Arctic cod fed different diets: capelin fillets, *Calanus* copepods, and *Themisto* amphipods.

Experiment and year	Diet	*n*	Duration (days)	Fish weight (g)	Growth rate (mg·day^{-1})	Growth rate (%·day^{-1})	Daily ration (no.·day^{-1})	Daily ration (mg·day^{-1})	Daily faeces (mg·day^{-1})
Maximum ration									
1988	Capelin	3	56	3.99±1.99	53.2±21.3	1.487±0.162	—	130.1±66.8	0.739±0.169
1988	Capelin	4	56	26.29±2.32	150.4±18.1	0.577±0.057	—	408.5±27.6	1.454±0.227
1988	Capelin	7	56	59.18±4.01	146.6±19.1	0.253±0.032	—	647.7±65.7	2.139±0.182
1988	Capelin[a]	12	56	40.28±2.32	177.0±16.1	0.449±0.042	—	470.3	2.006
1988	Capelin[a]	12	28	41.90±2.30	241.2±20.0	0.588±0.046	—	560.8	1.151
1989	*Calanus*	5	48	4.93±0.63	56.3±5.7	1.179±0.105	10.15±1.26	98.5±12.2	1.174±0.090
1989	*Themisto*	5	48	4.40±0.40	38.0±3.9	0.908±0.142	5.51±0.18	112.9±3.7	5.130±1.564
1990	*Calanus*	8	36	11.21±0.46	62.6±8.8	0.551±0.064	17.93±1.65	168.0±14.8	2.772±0.666
Reduced ration									
1989	*Calanus*[b]	5	24	5.08±0.51	12.9±1.9	0.254±0.033	2.15±0.21	20.9±2.0	0.463±0.077
1989	*Calanus*[c]	5	24	5.39±0.54	–0.4±1.8	0.003±0.027	1.10±0.10	10.7±1.0	0.463±0.054
1989	*Themisto*[b]	5	24	4.14±0.63	8.2±1.9	0.221±0.063	2.14±0.20	43.8±4.1	0.994±0.120
1989	*Themisto*[c]	5	24	4.33±0.62	19.1±6.0	0.412±0.064	1.20±0.20	24.6±4.1	1.504±0.248
1990	*Calanus*	10	36	11.66±0.34	–10.9±5.2	–0.089±0.044	3.02±0.02	28.4±1.6	0.666±0.046
Starvation									
1988	None	12	20	47.46±2.47	–80.8±17.5	–0.173±0.033	0	0	0.386
1989	None	8	48	5.19±0.22	–7.1±1.0	–0.134±0.018	0	0	0
1990	None	10	36	11.99±0.41	–42.9±3.8	–0.357±0.028	0	0	0

Note: Maximum (satiation) and reduced (close to maintenance) rations were fed to individuals or groups of marked fish. Data are means ± SE for individual fish (*n*), with weights as wet weight except for faeces (dry weight).

[a] Group experiment.

[b] Two prey items daily.

[c] One prey item daily.

When rations were reduced, growth of large fish (>40 g) declined over time (Fig. 2*b*). Growth became zero or slightly negative after 28 days at a ration of 0.5% body weight·day^{-1}, indicating that this ration was close to maintenance (zero growth). The medium-sized fish (20–40 g) showed a similar pattern, although the growth changed little after day 28 even if rations were reduced (Fig. 2*b*). The smallest fish (<10 g) showed little change in growth with ration size, although these small rations (very small pieces of fillets) were difficult to weigh accurately, and as mentioned before the absolute growth rate of small fish was low.

In the group feeding experiment with capelin as food, fish grew somewhat faster (177–241 mg·day^{-1}) than in experiments with individuals (Table 1, Fig. 2*c*). Starved fish (group 2) lost 150 mg·day^{-1} during the first week, probably because stomach contents were still being digested, and then 81 mg·day^{-1} during the next 20 days (Table 1, Fig. 2*c*).

Growth was also affected by the type of food given. When given live Arctic zooplankton, small fish (5 g) fed maximum rations of *Calanus* had faster growth rates (56 mg·day^{-1}) than similarly sized fish fed *Themisto* (38 mg·day^{-1}) (Fig. 3*a*). Larger fish (11 g) fed maximum rations of *Calanus* had slightly higher absolute growth rates (63 mg·day^{-1}) and lower relative rates than the smaller fish (Table 1, Figs. 3*a* and 3*b*). Reduced rations were adjusted to approximate maintenance ration and gave close to zero growth (1989) or slight weight loss (1990). The starvation groups lost weight in both years, although both absolute and relative weight loss was greater in the 1990 (43 mg·day^{-1}, or 0.36%·day^{-1}) than in the 1989 experiment (7 mg·day^{-1}, or 0.13%·day^{-1}). Low weight loss in 1989 was likely due to an additional, although small, food source of zooplankton entering the system because of insufficient filtering of inflowing water (see Materials and methods).

Maximum daily rations

Maximum daily rations (MDR) of *Calanus* was 10 individuals (mean length 6.5 mm) for by small fish, and 18 individuals (mean length 5.0 mm) for larger fish, corresponding to daily rations of 99 and 168 mg, respectively (Table 1). Maximum daily ration of *Themisto* for small fish was 5.5 individuals (mean length 12 mm), corresponding to 113 mg, which was similar to the ration of *Calanus*. There was a significant relationship ($p = 0.0001$, $r^2 = 0.761$) between numbers of *Calanus* consumed (MDR_n) and fish weight for the 1989 and 1990 experiments combined (Fig. 4*a*). Given a significant relationship ($p = 0.0001$, $r^2 = 0.985$) between numbers (MDR_n) and weights (MDR_w) of *Calanus* consumed in 1990, MDR_w also changed with fish weight. However, only a limited size range of fish was used in the 1990 experiment (Fig. 4*b*). Therefore, to establish a significant ($p = 0.0001$) relationship between MDR_w and fish weight, we used ration weights of capelin fillets from the 1988 experiment (Fig. 4*b*).

Maintenance ration

Daily rations for fish fed reduced (near maintenance) rations were 10–44 mg·day^{-1} (Table 1). We determined the maintenance ration of *Calanus* by regressing mean daily ration against mean absolute growth rate (G_1) for individual fish (mean weight 12 g) in the 1990 experiment. Daily maintenance ration was 60.2 mg (583.7 J) of *Calanus* (Fig. 5*a*), which was equivalent to 5.7 individuals per day. This maintenance ration was equivalent to 0.5% body weight·day^{-1}, which

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Fig. 2. Absolute growth (change in wet weight) of Arctic cod in feeding experiments on individuals (*a* and *b*) and groups (*c*), 1988. Fish were fed chopped capelin fillets. Means (±SE) for growth of individual fish are indicated. (*a*) Maximum (daily feeding to satiation) rations for different size groups: <10 g ($n = 3$); 20–40 g ($n = 4$); >40 g ($n = 7$). (*b*) Reduced ration, a percent body weight ration that declined by half every 2 weeks (2, 1, 0.5, 0.25%), fed different size groups: <10 g ($n = 2$); 20–40 g ($n = 6$); <40 g ($n = 4$). (*c*) Two groups of fish ($n = 12$) fed maximum rations during the first 28 days. During the next 28 days, maximum rations were continued for group 1, while group 2 was starved. The two groups (1 and 2) had similar mean (±SE) weight at the start of the experiment (40.28 ± 2.32 g, 41.90 ± 2.30 g).

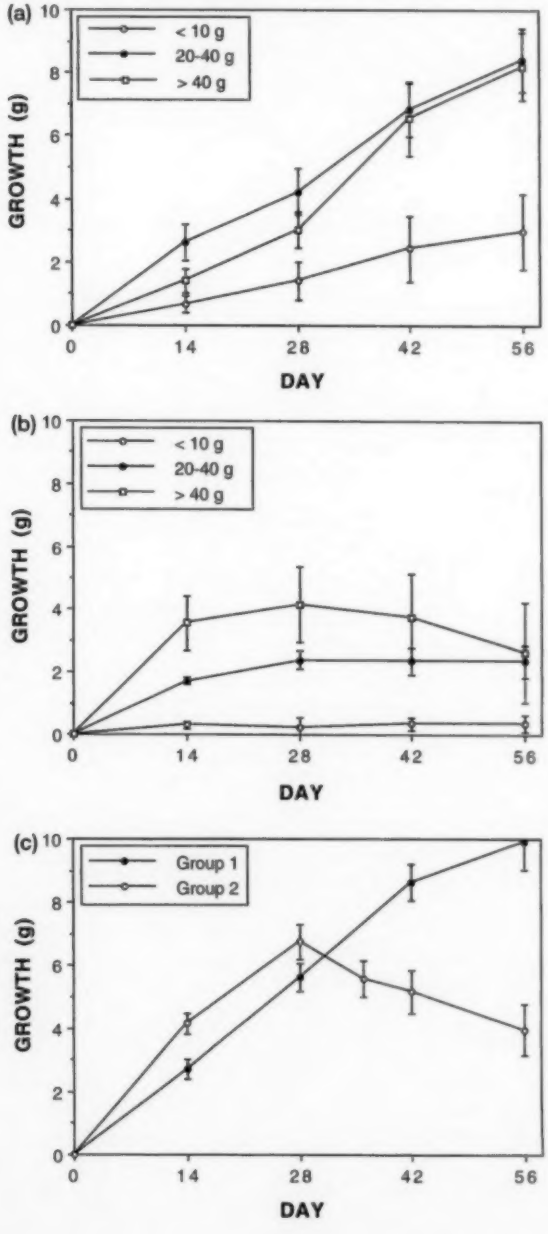
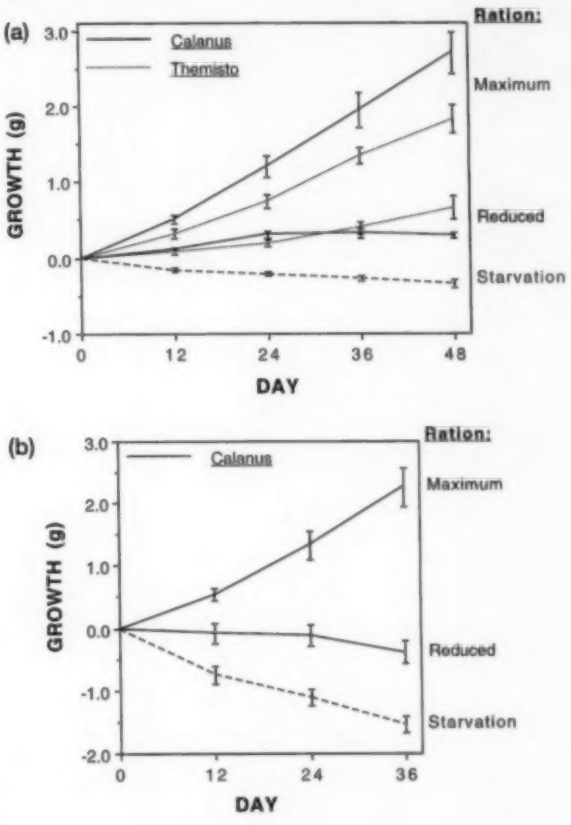


Fig. 3. Absolute growth (change in wet weight; mean ± SE) of Arctic cod in feeding experiments on individuals with three different rations: maximum (daily feeding to satiation), reduced (adjusted close to maintenance), and starvation (see Table 1 for information on ration sizes). (*a*) 1989 experiment, fish fed *Calanus* and *Themisto*: maximum rations ($n = 5$ each), reduced rations ($n = 5$ each), and starvation ($n = 8$). (*b*) 1990 experiment, fish fed *Calanus*: maximum ration ($n = 8$), reduced ration ($n = 10$), and starvation ($n = 10$).



was similar to that indicated in the experiment with declining rations over time (Fig. 2*b*). However, to also maintain the energy content of a 12-g Arctic cod, more energy (716.3 J) was needed (Fig. 5*b*), which corresponded to 73.8 mg (0.6% body weight) or 7.2 individual *Calanus* per day.

Energy contents

The energy content of food affected growth rates of small fish fed at maximum rations. The fastest growth rates resulted from *Calanus*, which had a higher energy and lipid content than capelin fillets and *Themisto* (Table 2). *Themisto* contained less energy partly because of its thicker exoskeleton. The energy contents of male and female Arctic cod were similar and slightly higher than that of juveniles (Table 2). Arctic cod store energy as lipids (mainly triglycerides) in the liver (Table 2), but because carcass (low in lipids) constituted a much higher proportion (77.5%) of the total weight than liver (9.2%), the total lipid content of each fish was relatively low.

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Fig. 4. Maximum daily rations (MDR) presented as (*a*) numbers (MDR_n) or (*b*) weight (MDR_w) of copepods and capelin fillets, consumed over 12–14 day periods, as a function of wet weight (W) of Arctic cod: (*a*) $\log(MDR_n) = 0.9191\log(W) + 0.2608$, $r^2 = 0.761$; (*b*) $\log(MDR_w) = 0.7138\log(W) + 1.5272$, $r^2 = 0.896$. MDR_w of *Calanus* is plotted for comparison, but not included in the regression.

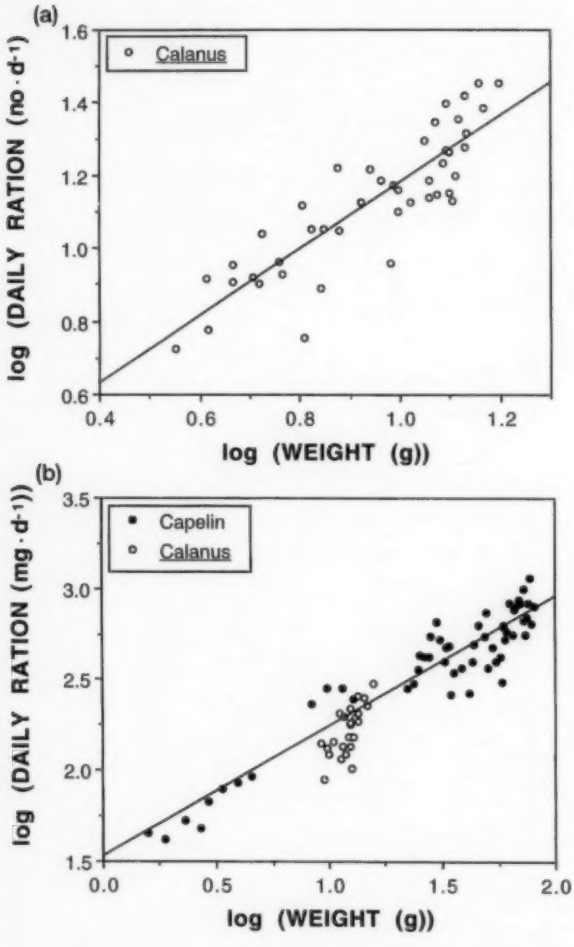
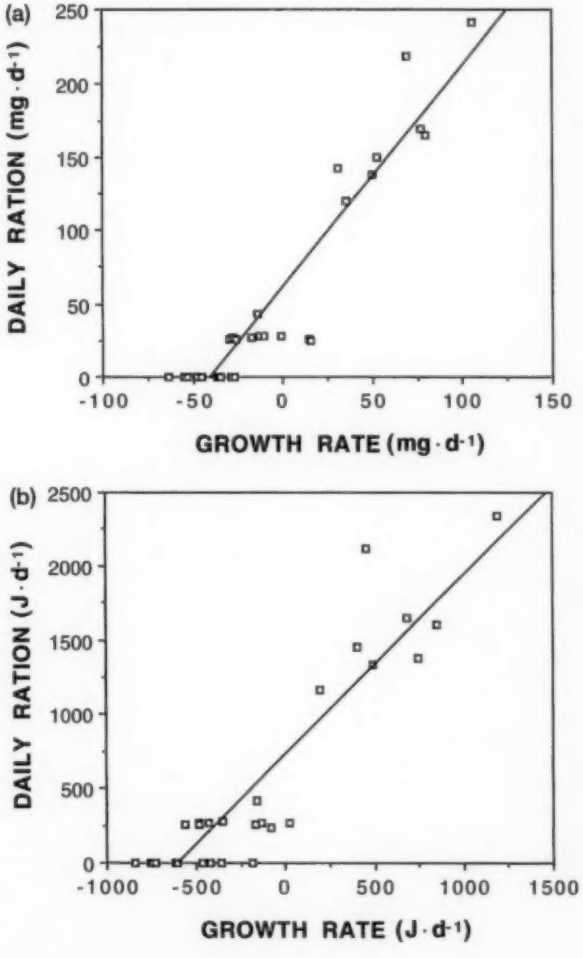


Fig. 5. Mean daily ration (DR) against growth rate (G_1) for Arctic cod, in feeding experiment 1990: (*a*) Weight of daily ration (DR_w) regressed against growth rate: $DR_w = 60.163 + 1.5120G_1$, $r^2 = 0.890$. (*b*) Energy of daily ration (DR_e) regressed against growth rate, including change in energy content of experimental fish: $DR_e = 716.32 + 1.2117G_1$, $r^2 = 0.851$.



The energy content of faecal matter was highest for capelin and lowest for *Themisto*, while energy contents of faecal matter from wild fish were intermediate between faeces from *Calanus* and *Themisto* diets (Table 3). The amount of dry faecal matter recovered daily in experiments varied between 0.7–5.1 mg·day^{-1} (0.09–0.52% body weight) with *Themisto* diets giving the highest daily amounts because of poorly digested chitinous exoskeletons (Table 1). Faecal matter from zooplankton diets contained mostly protein (lipid/protein ratio 0.1–0.5), indicating that lipids were predominately assimilated. Faecal matter from capelin diets contained relatively more lipids (lipid/protein ratio 3.3), and therefore had higher energy content (Table 2), which indicated less assimilation of capelin lipids.

When fish were fed to satiation, they increased in weight and in energy content; both have to be considered when accounting for energy assimilated into growth. Fish fed on capelin diets added weight but lost energy from tissues, whereas fish fed *Calanus* added energy content, and fish fed *Themisto* changed little (Table 4). Fish fed reduced diets and starved individuals generally lost energy from tissues.

Energy budgets

Total energy budgets ($N = 8$) for experiments with maximum rations, 1988–1990, accounted for 72–104% of the energy in balanced budgets ($(G + R + F + U)/C \times 100$) (Table 5, Fig. 6). Growth contributed most to the budget of small fish fed high energy diets of *Calanus*, whereas respiration contributed most to the budgets of larger fish fed low energy diets of capelin. An average energy budget ($N = 3$; 1989 and 1990 budgets combined:

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Table 2. Dry/wet weight, energy, lipid, and protein contents in samples (*n*) of prey and Arctic cod.

Source	*n*	Dry/wet weight (%)	Energy content (kJ·g dry wt.$^{-1}$)	Energy content (kJ·g wet wt.$^{-1}$)	Lipids (% dry wt.)	Lipids (% wet wt.)	Protein (% dry wt.)	Protein (% wet wt.)
Prey								
Capelin	7	20.65±0.35	24.404±0.278	5.039±0.057	16.90±2.05	3.28±0.40	71.46±2.01	13.86±0.39
Calanus	5	31.29±1.48	31.008±0.451	9.702±0.141	50.41±1.26	16.82±0.42	31.03±0.89	10.35±0.30
Themisto	6	19.42±1.99	21.086±1.225	4.095±0.238	25.72±1.62	5.61±0.35	39.55±1.21	8.63±0.26
Arctic cod								
Juveniles	15	21.76±0.31	25.539±0.187	5.557±0.041	23.94±0.76	5.39±0.17	55.32±1.11	12.47±0.25
Adults	100	24.71±0.15	26.140±0.092	6.459±0.023	33.53±0.42	8.29±0.10	44.93±0.35	11.10±0.09
Males	50	24.54±0.22	26.138±0.135	6.414±0.033	34.36±0.59	8.43±0.14	44.31±0.47	10.87±0.12
Females	50	24.89±0.20	26.141±0.126	6.506±0.031	32.70±0.59	8.14±0.15	45.55±0.51	11.34±0.13
Male tissues								
Carcass	7	19.68±0.24	21.504±0.331	4.232±0.065	14.25±0.83	2.80±0.16	62.4±0.54	12.28±0.11
Liver	7	73.14±3.04	37.011±0.259	27.070±0.189	83.82±1.54	63.00±1.16	5.33±0.73	4.00±0.55
Intestine	7	27.52±0.71	26.721±0.458	7.354±0.126	23.43±2.73	6.42±0.75	36.23±1.63	9.93±0.45
Gonad	7	32.32±1.16	30.414±0.437	9.830±0.141	48.91±5.38	14.59±1.60	35.71±4.37	10.66±1.30
Female tissues								
Carcass	7	20.05±0.44	22.037±0.276	4.418±0.055	12.87±0.83	2.56±0.16	64.37±0.87	12.80±0.17
Liver	7	68.92±3.94	35.840±0.443	24.701±0.305	79.70±1.95	61.42±1.51	5.63±0.97	4.33±0.74
Intestine	7	29.07±1.14	27.057±0.565	7.865±0.164	28.05±2.45	7.69±0.67	34.85±1.96	9.55±0.54
Gonad	7	28.09±1.08	28.580±0.850	8.028±0.239	44.93±4.38	13.54±1.32	38.74±3.28	11.67±0.99

Note: Values are mean ± SE. Values for juveniles included whole fish, whereas values for adults were calculated based on wet weight contributions and respective energy, lipid, and protein contents of four tissues (carcass, liver, intestine, and gonad) Fish samples were frozen (prior to dissection) and stomach contents were excluded.

Table 3. Energy content of faecal matter samples (*n*) collected from fish in the 1988–1990 experiments and from groups of juvenile and adult fish fed specific diets for about 4 weeks prior to sampling.

Experiment and year	Diet	*n*	Energy content (kJ·g dry wt.$^{-1}$)
Maximum ration			
1988	Capelin	2	25.214
1989	*Calanus*	1	11.443
1990	*Calanus*	1	16.670
Reduced ration			
1990	*Calanus*	1	9.090
Group collections			
Juveniles (1989)	*Calanus*	1	11.633
Adults (1989)	*Calanus*	1	7.446
Juveniles (1989)	*Themisto*	1	4.649
Adults (1989)	*Themisto*	1	4.761
Juveniles and adults (1989)	Unknown	1	8.507
Juveniles (1991)	Unknown	2	6.910

Note: Faecal matter from wild fish (diets unknown) were sampled within 2 days of capture.

Table 5) for juvenile Arctic cod fed maximum rations of Arctic zooplankton (Fig. 7) was $100C = 50G + 33R + 3F + 6U + 8Z$, where $R = 24R_1 + 9SDA$, based on an average value of 37.5% SDA from Hop and Graham (1995), R_1 is then routine respiration, and Z is activity and other unaccounted energy.

In the 1990 experiment we estimated the maintenance ration ($G = 0$) to be 716.3 J·day^{-1} (Fig. 5*b*). If we use the 1990 values for reduced ration, with F as a fraction of the estimated maintenance ration, we get $100C = 41R + 2F + 7U + 50Z$, which implies that active metabolism may account for 50% of C or 358 J·day^{-1}. If this value is added to the 1990 budget for maximum ration the balance becomes 93.6%.

Growth and assimilation efficiencies

Gross (GGE) and net (NGE) growth efficiencies varied considerably in experiments (Table 5). Values for the average budget above were 50%, and 60%, respectively (lower if active metabolism is included). Assimilation efficiencies (AE) were generally lower for capelin than for Arctic zooplankton and higher for group experiments involving capelin than in experiments on individuals (Table 5). A mean value for the combined budget was about 80%, although values in different experiments on maximum rations varied from 64 to 91% (Table 5).

Discussion

Arctic cod feed primarily on planktonic copepods and amphipods, ice-associated amphipods, and mysids (Bain and Sekerak 1978; Craig et al. 1982; Bradstreet et al. 1986; Lønne and Gulliksen 1989). However, the energy budget for Arctic cod at low temperatures ($100C = 50G + 33R + 3F + 6U + 8Z$) differed substantially from a generalized budget for carnivorous fish: $100C = 29G + 44R + 20F + 7U$ (Brett and Groves 1979), and it also differed from the energy budget of an Antarctic fish (*N. neglecta*): $100C = 6G + 74R + 20(F + U)$ (Everson 1970). The AE for Arctic cod was about 10% higher than the generalized budget, but it was similar to the Antarctic budget (80%), probably because assimilation efficiency increases with decreasing temperature (Elliott 1976). However, the GGE was much higher for Arctic cod (50%) than for both the generalized budget (29%) and the Antarctic fish budget (6%). In particular the Antarctic fish allocated a much higher proportion of the energy to respiration, which was attributed by Everson (1970) to metabolic cold adaptation (Wohlschlag 1960), although Holeton (1974) questioned whether this phenomenon was real or a stress-induced artifact.

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Fig. 6. Energy budgets ($N = 8$) for experimental groups fed maximum rations. The 1988 experiments included capelin diets fed to three size-classes of individual fish (<10, 20–40, >40 g) and two groups of fish (*, fed to satiation during the whole period; **, fed to satiation during the first half of the period and subsequently starved). The 1989 and 1990 experiments included individuals fed *Calanus* or *Themisto*. Components of energy budgets are expressed as a percentage of total energy ingested (a fully balanced budget adds to 100%).

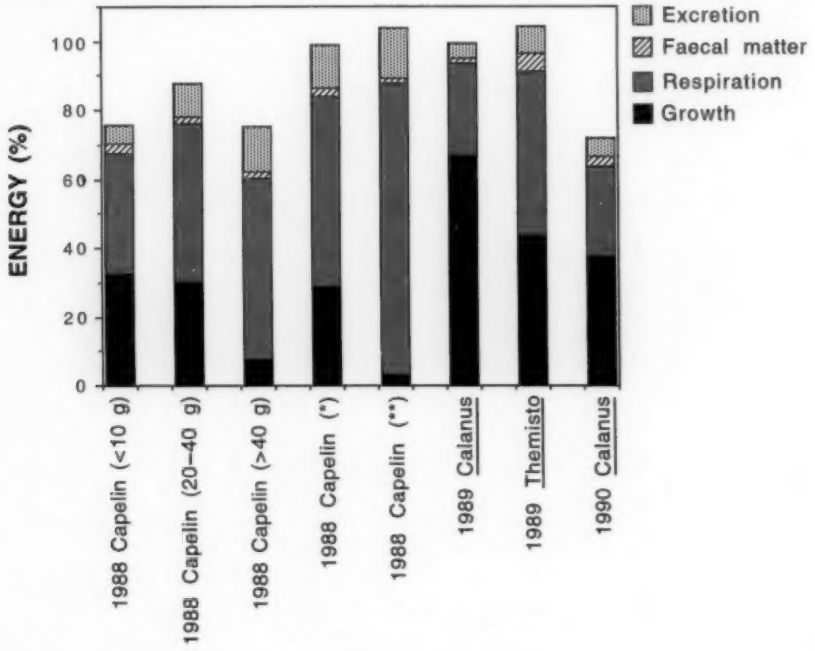

Table 4. Dry/wet weight and energy content of fish (n) at the end of feeding experiments.

Experiment and year	Diet	n	Duration (days)	Initial fish weight (g)	Final fish weight (g)	Dry/wet weight (%)	Energy content (kJ·g dry wt.$^{-1}$)	Energy content (kJ·g wet wt.$^{-1}$)	Δ Energy content (kJ·g wet wt.$^{-1}$)
Maximum ration									
1988	Capelin	3	56	3.99±1.99	6.97±3.18	20.66±0.54	23.180±0.334	4.655±0.189	–0.451
1988	Capelin	4	56	26.29±2.32	34.72±3.00	23.12±0.33	24.059±0.033	5.563±0.086	–0.448
1988	Capelin	7	56	59.18±4.01	67.39±4.38	22.70±0.65	24.035±0.112	5.460±0.175	0.523
1988	Capelin[a]	12	56	40.28±2.32	50.19±2.77	23.27±0.16	24.133±0.082	5.615±0.051	–0.427
1988	Capelin[b]	12	56	41.90±2.30	45.84±2.50	23.12±0.19	24.006±0.097	5.550±0.058	–0.456
1989	*Calanus*	5	48	4.93±0.63	7.63±0.84	27.37±0.54	27.841±0.100	7.618±0.149	2.061
1989	*Themisto*	5	48	4.40±0.40	6.22±0.31	22.21±0.57	24.717±0.505	5.501±0.250	–0.056
1990	*Calanus*	8	36	11.21±0.46	13.46±0.70	23.70±0.52	26.415±0.244	6.269±0.195	0.712
Reduced ration									
1989	*Calanus*	5	48	5.08±0.51	5.38±0.50	22.86±0.45	25.135±0.255	5.750±0.160	0.193
1989	*Thamisto*	5	48	4.14±0.63	4.79±0.76	20.34±0.54	23.238±0.457	4.735±0.216	–0.822
1990	*Calanus*	10	36	11.66±0.34	11.27±0.31	20.25±0.42	23.852±0.328	4.842±0.167	–0.715
Starvation									
1989	None	8	48	5.19±0.22	4.85±0.20	21.63±0.50	24.459±0.357	5.301±0.197	–0.256
1990	None	10	36	11.99±0.41	10.44±0.37	18.84±0.69	22.985±0.436	4.358±0.240	–1.199

Note: Values are mean ± SE. Energy content of individuals in the 1988 experiment was calculated, based on wet weight contributions and respective energy contents of four tissues (carcass, liver, intestine, and gonads). Change in energy content of experimental fish (relative to fish in Table 2) is indicated; in the 1988 experiments half of the energy loss was assumed to have occurred before experiments started (1.5 months from time of capture). Fish samples were frozen (prior to dissection) and stomach contents were excluded.

[a]Group fed to satiation during the whole period.

[b]Group fed satiation during the first half of the period and subsequently starved.

Our budgets for fish fed maximum rations accounted for 72–104% of the energy, which is comparable with other budgets reported in the literature (e.g., Brafield 1985). However, most reported budgets are not complete, some parameters being estimated by difference or proportion. Energy budgets for reduced rations were considerably off balance except for the *Themisto*-fed fish (112%). In these budgets, consumption values were low, and small errors in the determination of this

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Fig. 7. Energy budget ($N = 3$) for Arctic cod fed Arctic zooplankton: $100C = 50G + 24R_1 + 9SDA + 3F + 6U + 8Z$, where Z was activity and unaccounted energy.

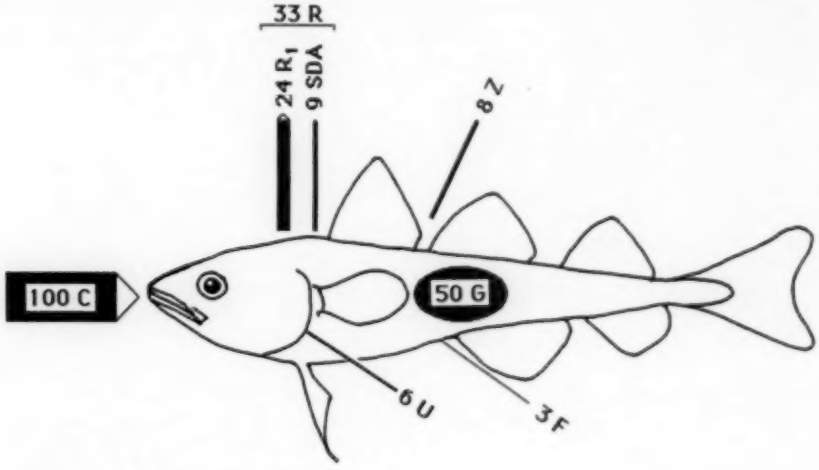

Table 5. Mean daily energy budget ($J \cdot day^{-1}$) in controlled feeding experiments on Arctic cod, 1988–1990.

Experiment and year	Diet	*n*	Duration (days)	Fish weight (g)	Consumption ($J \cdot day^{-1}$)	Growth ($J \cdot day^{-1}$)	Respiration ($J \cdot day^{-1}$)	Faeces ($J \cdot day^{-1}$)	Excretion ($J \cdot day^{-1}$)	Balance (%)	GGE (%)	NGE (%)	AE (%)
Maximum ration													
1988	Capelin	3	56	5.48	655.6	215.6	226.2	18.6	36.6	75.8	32.88	48.80	67.39
1988	Capelin	4	56	30.51	2058.4	627.1	940.8	36.7	203.8	87.9	30.47	40.00	76.17
1988	Capelin	7	56	63.29	3263.8	247.8	1724.1	53.9	422.8	75.0	7.59	12.57	60.42
1988	Capelin[a]	12	56	45.24	2369.8	686.5	1304.7	50.6	302.2	98.9	28.97	34.48	84.02
1988	Capelin[b]	12	56	46.26	1412.9	49.3	1191.0	19.4	208.2	103.9	3.49	3.97	87.78
1989	*Calanus*	5	48	6.28	955.6	640.2	256.2	13.4	42.0	99.6	66.99	71.42	93.80
1989	*Themisto*	5	48	5.31	462.3	203.4	218.0	23.8	35.5	104.0	44.00	48.27	91.16
1990	*Calanus*	8	36	12.34	1629.9	613.5	426.1	46.2	82.4	71.7	37.64	59.01	63.78
Reduced ration													
1989	*Calanus*	5	48	5.23	153.3	56.4	157.6	4.2	23.8	157.8	—	—	—
1989	*Themisto*	5	48	4.47	140.0	–6.8	137.3	5.8	20.3	111.8	—	—	—
1990	*Calanus*	10	36	11.47	275.5	–284.0	291.9	6.1	52.1	24.0	—	—	—
Starvation													
1989	None	8	48	5.02	0	–65.2	153.7	0	12.1	39.3	—	—	—
1990	None	10	36	11.22	0	–587.0	289.1	0	27.0	185.7	—	—	—

Note: Fish weight is mean of initial and final weight (Table 4). Consumption (C) is calculated from daily ration and energy content of food (Tables 1 and 2). Growth (G) is calculated from weight increase and change in energy content of experimental fish (Table 4). Respiration (R) is calculated from values of starved and fed fish, adjusted for temperature and fish size (Hop and Graham 1995). Egestion (F) is calculated from daily faeces and their energy contents (Tables 1 and 3). Excretion (U) was determined separately as 13.58 and 4.90 μg ammonium$\cdot g^{-1} \cdot h^{-1}$ for fed and starved fish, respectively (we used an intermediate value of 9.24 for reduced rations). Coefficients for conversion of oxygen consumption and ammonia to energy equivalents were used (see Materials and methods). Balance is $(G + R + F + U)/C \times 100$. Gross (GGE) and net (NGE) growth efficiencies, and assimilation efficiency (AE) are indicated (see Materials and methods).

[a] Group fed to satiation during the whole period.

[b] Group fed to satiation during the first half of the period and subsequently starved.

or other components would tend to throw the budgets off balance. Budgets for starved individuals also did not balance. In the 1989 experiment, weight loss was probably less than it should have been as fish fed on small zooplankton passing through the filtering system. This may also have affected the reduced ration budgets of that year but probably had little effect on the maximum ration budgets. Weight loss values for the 1990 experiment were the most accurate because the improved filter system did not allow zooplankton to pass through.

In our energy budgets, consumption and growth were probably determined most accurately. Consumption was determined as both numbers and weight of prey. Weighing out daily individual rations was very labor intensive but gave better results than just numbers and sizes of prey, which was the reason why this was done in the 1990 experiment. Changes in energy content of the live zooplankton prey should also have been monitored as their energy content is dependent on several factors that vary seasonally (e.g., lipid content and composition,

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stages). However, our experiments were conducted over a relatively short time period, so the effect of the seasonal signal was probably relatively small.

The values given for consumption represent short-term estimates, and field values may be quite different. The maximum daily ration in the laboratory is probably higher than that of Arctic cod in the wild, where food intake is also affected by food availability and fish behavior. A separate study showed that, although dispersed Arctic cod generally feed, schooling cod had high frequencies of empty stomachs (65% on average) (Hop et al. 1997). The mean stomach fullness of dispersed fish was 32% of the maximum possible (Hop 1994). Fish fed to satiation in the 1990 experiment had similar mean stomach fullness (30% of maximum) when dissected after the experiment. This may indicate that nonschooling fish may feed near sustained satiation level, on average, even if stomachs are less than full.

Estimated daily rations of Arctic cod, determined in gastric evacuation experiments (Hop 1994), resulted in values similar to those of this study. Juveniles (mean weight 6.6 g) fed *Calanus* and *Themisto* evacuated about 0.22 g·day^{-1} within the first 24 h and 0.14 g·day^{-1} at half-time (when half of the contents of a full stomach were evacuated). Because fish fed to satiation had less than full stomachs, daily ration values in our study are probably closer to daily rations estimated from half-time evacuation values (Hop 1994). If the daily ration of Hop (1994) is adjusted for fish weight (to 5.8 g, the mean weight of fish in the 1989 experiment) based on the relationship between MDR_w and fish weight (Fig. 4*b*), the resulting value (0.12 g·day^{-1}) becomes the same as our 1989 estimate of daily rations of Arctic zooplankton (0.11 g·day^{-1}). Daily rations, determined from gastric evacuation half-time values for larger fish (0.4–0.5 g·day^{-1}) were also similar to our values for large fish (0.5 g·day^{-1}). Jensen et al. (1991) found that daily rations for Arctic cod (10–34 g), fed peeled shrimp (4.31 kJ·g^{-1}) to satiation four times per week, varied from 0.12 to 0.22 g·day^{-1} during periods of low (January–February) and high (September–December) food intake. Their high value is about half of our daily ration value for Arctic cod (26 g) fed capelin, indicating that their fish fed below satiation level.

Different diets will be evacuated differentially (Jobling 1987), and this may introduce some errors into the determination of MDR_w. The question concerning our experiments is whether chopped capelin fillets were digested faster or slower than *Calanus* copepods. Other factors being equal, the rate of digestion and fragmentation of large food items, such as capelin fillets, would be expected to be slower than that of the same volume of food composed of a number of smaller particles (Jobling 1987). However, high energy content combined with exoskeleton, in the case of *Calanus* copepods, also retards digestion. Given the very slow evacuation rates observed for a *Calanus* diet (Hop 1994), it is doubtful that a capelin diet is evacuated even slower, which would be the prediction. Daily rations in our experiments are therefore mainly a function of fish size rather than diet type, which was indicated above by the similarity in the outcomes. However, the effects of ration type should be further evaluated experimentally, because other feeding experiments involving Arctic cod have used different unnatural food sources (Jensen et al. 1991; Christiansen 1995: Christiansen and George 1995).

Growth during the experiments was determined as changes in both body mass and energy content. Both dry/wet weight ratios and energy content of Arctic cod used in our experiments were similar to those reported by Brekke and Gabrielsen (1994) from Spitsbergen waters, but the energy content of the experimental fish changed during experiments. It became apparent that fish fed capelin diets lost energy from tissues when they grew whereas fish fed *Calanus* increased in caloric content. This was probably mainly because of the much lower lipid (and energy) content of the capelin diet as compared with the *Calanus* diet, and the lipid content of capelin fillets was also lower than that of the Arctic cod itself. Lipids are the most important energy reserves in Arctic food chains (Falk-Petersen et al. 1990), but it is not possible to build up such reserves on a lipid-poor diet. In addition, the type of lipid involved is important. Arctic *Calanus* sp. contain high amounts (>80%) of wax esters (Lee 1974), whereas capelin contains mainly triglycerides (Montevecchi and Piatt 1984). The relatively high lipid content in faeces indicated poorer assimilation of capelin lipids than *Calanus* lipids, which may be because the digestion enzymes are better able to process a natural diet.

Even though growth was a main component of the energy budget for Arctic cod, it was slow, resulting from low daily food intake. Relative growth rates, in our experiments with maximum rations, ranged from 0.6 to 1.5% body weight·day^{-1}, depending on fish size. However, the growth rate of wild fish was considerably less, about 0.25% body weight·day^{-1} between age 1 and 2, and 0.15%·day^{-1} between age 2 and 3 (from size-at-age relationship; Hop et al. 1997). In an enclosure experiment (10-m^3 cage) with age-1 Arctic cod (n = 12, mean weight 6.7 g) in Resolute Bay, the mean growth rate during August was 0.45% body weight·day^{-1} (H. Hop, unpublished data), which was higher than the annual growth rate but lower than growth rates for similarly sized fish fed maximum rations in the laboratory (1.0% body weight·day^{-1}).

Our respiration values were short-term acclimated VO_2 values determined for juvenile Arctic cod (Hop and Graham 1995), which were 70–90% above long-term acclimated (>5 months) values (Hop and Graham 1995). We included feeding metabolism (SDA) in our values but not active metabolism. Our estimate of active metabolism, based on maintenance rations in the 1990 experiment, indicated that our respiration values were too low, which would partly explain why some of the budgets did not add up to 100%. Even if fish were confined to individual boxes and activity was low, they were disturbed daily during cleaning and feeding, which would elevate respiration rates. In a field situation, the repiration component will probably be higher because of swimming activities associated with feeding behavior and predator avoidance. The Arctic cod is generally a slow-swimming fish both in the laboratory and when schooling in nature (Graham and Hop 1995), and handling stress is absent in a field situation. Therefore, the field addition to the respiration budget is probably small. Future studies should determine field metabolic rates for the species, because such data are currently unavailable.

Faecal matter, contained in mucous membranes, was easily collected daily from each compartment, and we probably recovered most of it. We did not account for degradation of faecal material while it was in the water (maximum 1 day), and thus our values are probably too low, but this process may have been slow at these low temperatures as most faecal matter

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seemed to be intact strings. Excretion values may also have been underestimated, since they were determined for confined animals at rest.

Clarke (1983) concluded that low energy intake in polar invertebrates, as compared with temperate ones, may be an adaptation to cold water. In a typical polar marine invertebrate, growth is slow and seasonal, basal metabolism low, growth efficiency high, annual individual reproductive effort reduced, and the cost of activity about the same when compared with related warmer water species. Our energy budgets indicate a similar pattern for an Arctic fish. Low energy intake for wild Arctic cod probably partly results from low food availability. However, low daily rations for fish fed to satiation indicates that slow rates of gastric evacuation may limit food intake when food is abundant (Hop 1994).

Arctic cod has been described as an r-selected species (Craig et al. 1982; Mikhail 1985), because it is a small (<30 cm fork length), relatively short lived (maximum life-span of 7 years; very few live past 5 years) fish that reaches maturity at an early age (2–3 years) (Bain and Sekerak 1978; Craig et al. 1982; Bradstreet et al. 1986). Mortality rates are high with relatively few fish surviving past 4 years (Welch et al. 1993; Hop et al. 1997). However, some life-history aspects, such as slow growth and relatively low fecundity (Andiyashev 1954; Hop et al. 1995), do not fit an r-selected pattern (Adams 1980).

Growth of Arctic cod is not directly comparable with other gadids living at higher temperatures. The growth rate of Arctic cod (Hop et al. 1997) is slower than Greenland cod (*Gadus ogac*) in Hudson Bay (Mikhail and Welch 1989), a K-selected species with slow growth (Mikhail 1985). The geographic range of Greenland cod overlaps partially with Arctic cod on the coasts of northern Labrador, Baffin Island, and Greenland; however, the growth rate of Arctic cod off the coast of Labrador (Lear 1983) remained below that of slow-growing Greenland cod in Hudson Bay (Mikhail and Welch 1989). However, the growth rate of Arctic cod was higher than that of sympatric benthic species, such as snailfishes (Falk-Petersen et al. 1988).

Arctic cod had high growth and assimilation efficiencies at low temperatures, which means that they are very efficient converters of energy from lower (zooplankton) to higher trophic levels (marine mammals and sea birds). However, the growth of individual fish was slow owing to low food intake. The relatively high annual production of Arctic cod therefore results from high growth efficiency coupled with large standing biomass.

The annual production of Arctic cod must at least equal the annual predation (148 000 t). If one assumes that the mean annual growth rate of the standing biomass of Arctic cod is about 0.5 (based on a mean growth rate of 0.15%·day^{-1} of fish between age 2 and 3) the standing biomass must be at least 300 000 t in the Lancaster Sound Region or five times the biomass estimate of Welch et al. (1992). In comparison, acoustic surveys from the Barents Sea have estimated the stock size of Arctic cod to be approximately 1 million tons (Gjøsæter 1993).

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Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*

Chris J. Foote, Gayle S. Brown, and Chris C. Wood

Abstract: Protein electrophoresis was used to determine the relative spawning success of jack sockeye salmon, *Oncorhynchus nerka*, males when they joined spawnings of larger, older individuals in experimental enclosures in the natal stream, Pierre Creek, Babine Lake, British Columbia. We attempted to relate the results to distance of the jack from female before spawning and relative size of the jack to the alpha male. Further, we measured the relationships between relative gonad size and available sperm volume to body size to determine if sperm competition could also play a role in determining the relative spawning success of males. Jack spawning success was variable (3–93%) and not significantly different from that of large males, even though they were further from the female before spawning, had smaller gonads, and had less available sperm. Jack males were adept at sneaking, usually commencing spawning within a split second of the alpha male and always on the opposite, free, side of the spawning female. The results are related to various theories on the evolution of alternative mating strategies and tactics in Pacific salmon.

Résumé : L'électrophorèse des protéines a été utilisée pour déterminer le succès de reproduction relatif de jeunes mâles précoces de saumon sockeye, *Oncorhynchus nerka*, lorsqu'ils se joignent d'individus plus âgés et de plus grande taille pour frayer dans des enclos expérimentaux dans le cours d'eau natal, soit le ruisseau Pierre, lac Babine, Colombie-Britannique. Nous avons tenté de relier les résultats à la distance entre le jeune mâle précoce et la femelle avant le frai et à la taille relative du mâle précoce par rapport à celle du mâle alpha. De plus, nous avons mesuré la relation entre la taille relative des gonades et le volume de liquide spermatique disponible par rapport à la taille corporelle pour déterminer si la compétition entre les spermatozoïdes pouvait également jouer un rôle dans la détermination du succès de reproduction relatif des mâles. Le succès de reproduction des jeunes mâles précoces était variable (3–93%) et n'était pas différent de manière statistiquement significative de celui des mâles de plus grande taille, même s'ils étaient à une plus grande distance de la femelle avant le frai, avaient des gonades plus petites et avaient une moins grande quantité de spermatozoïdes disponibles. Les jeunes mâles précoces étaient habiles pour se faufiler, commençant habituellement à frayer à une fraction de seconde près par rapport au mâle alpha, et toujours du côté opposé, et libre, de la femelle en frai. Les résultats sont reliés à diverses théories sur l'évolution de diverses stratégies et tactiques de reproduction chez le saumon du Pacifique.
[Traduit par la Rédaction]

Introduction

Alternative mating behaviours in fishes have attracted much interest in recent years (e.g., Dominey 1984; Gross 1985; Kodric-Brown 1986; Jennings and Philipp 1992; see reviews by Taborsky 1994; Gross 1996). Topics have ranged from the discovery and description of behaviours and the conditions that evoke and shape them (e.g., Kodric-Brown 1988; Sigurjónsdóttir and Gunnarsson 1988; Magnhagen 1995), to morphological comparisons of different behavioral types (Gross 1984, 1991*a*; Noakes et al. 1995), to the relative breeding success of the different tactics involved (e.g., Hutchings and Myers 1988; Zimmerer and Kallman 1989), to the possible factors that have led to their evolution (e.g., Dominey 1984; Myers 1986; Holtby and Healey 1990; Bohlin et al. 1990; Gross 1991*a*, 1991*b*).

The existence of alternative mating behaviours has been long known in salmonids (e.g., Needham and Taft 1934; Jones and King 1952; Hanson and Smith 1967). In Pacific salmon, genus *Oncorhynchus*, the female selects and defends a territory in which she constructs and spawns in series of nests. She is often attended by multiple males, which may be of different sizes, age-classes, and life-history types. The largest male typically wins contests for close access to the female, with the other males relegated to positions downstream and (or) lateral to the pair. From these satellite positions, the subdominant males "sneak" in the spawning of the pair and release sperm near simultaneously with that of the dominant male and the release of eggs by the female (Gross 1985; Foote 1990). This area received renewed interest when Schroder (1981, 1982) and Chebanov et al. (1983) demonstrating actual breeding success of males adopting a sneaking tactic. Gross (1985) and Myers (1986) examined evolutionary mechanisms that might maintain alternative life histories in populations. Since then, the ability of sneaking males to fertilize eggs has been demonstrated in

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C.J. Foote.[1] School of Fisheries, Box 357980, University of Washington, Seattle, WA 98195, U.S.A.
G.S. Brown. School of Fisheries, Box 357980, University of Washington, Seattle, WA 98195, and Northwest Biological Science Center, National Biological Service, 6505, NE 65th Street, Seattle, WA 98115, U.S.A.
C.C. Wood. Pacific Biological Station, Canadian Department of Fisheries and Oceans, Nanaimo, BC V9R 5K6, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: cfoote@fish.washington.edu

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miyabe charr, *Salvelinus malma miyabei* (Maekawa and Onozato 1986), and in mature Atlantic salmon parr, *Salmo salar*, both in controlled conditions and in the wild (Hutchings and Myers 1988; Jordan and Youngson 1992).

In Pacific salmon, Schroder (1982) demonstrated that chum salmon, *O. keta*, males relegated into satellite positions by the presence of larger males were able to fertilize anywhere from 0 to 46% of the eggs laid. Similarly, Chebanov et al. (1983) showed that "sneaking" sockeye, *O. nerka*, males were also able to fertilize some of the eggs laid. Schroder's (1981, 1982) results, and his speculation that the satellite proportion of eggs fertilized was negatively related to the distance the male was from the female immediately prior to spawning, were critical in Gross's (1985) determination that the existence of jacks in coho salmon, *O. kisutch*, was the result of a negative frequency-dependent mixed evolutionarily stable strategy.

While the tactic of sneaking has been shown to be successful in Pacific salmon, the breeding success of true jack males has not yet been examined. Jack males are those that mature after growth at sea but at a young age without a matching female age- and size-class and are thus generally relegated to a sneak tactic by larger, older males (e.g., Foote and Larkin 1988; Gross 1991*a*). In chum salmon there are no true jacks and hence no clear sex-based alternative breeding strategies. Rather, the tactics of sneaking or acting as a consort to a female are likely components of a single conditional strategy in which males adopt different tactics depending on conditions such as the presence of competing males, the relative size of competitors if present, and the operational sex ratio in the stream (Schroder 1982). Jacks are common in sockeye salmon, but no jack males were involved in the early study of Chebanov et al. (1983). Therefore, examinations to date involve the success of tactics employed by males within a single conditional strategy, rather than a comparison of different strategies as Gross (1985) envisioned for coho salmon.

Factors involved in determining spawning success of subordinate males in salmonids are likely more complex than initially envisioned. The relationship between distance from the female and male spawning success remains undocumented as Schroder (1982) did not present any data or statistics to support his observation. Further, Hutchings and Myers (1988) summarized data on sneak success in salmonids and concluded that sneak success is likely a function of the relative size of the sneak to the dominant male. Finally, Olsén and Liley (1992) and Liley et al. (1993) demonstrated that the amount of free-flowing sperm in male salmonids is positively related to how physically close they are to the female prior to spawning, even when males of a similar size are involved. Hence, at this point we do not have estimates of actual jack spawning success in Pacific salmon, and the relationship between distance and sneak success needs elucidation, as well as the potentially interacting effects of size and available sperm.

Sockeye salmon spawn in creeks draining into Babine Lake, in British Columbia, Canada, during August (Hanson and Smith 1967; McCart 1970; Foote and Larkin 1988). Kokanee, the nonanadromous form of sockeye, spawn coincidentally with sockeye in these creeks and both jacks and kokanee males are commonly observed acting as sneaks to spawning sockeye pairs. Sneaking behavior by larger sockeye males, while observed, is not common (Foote and Larkin 1988).

In this study, we estimated the spawning success of jack sockeye males when they spawned with sockeye pairs and sometimes kokanee males in experimental enclosures in their natural environment. These results were related to the distance of the male from the female immediately before spawning commenced and to the relative gonad sizes and available sperm volume of participating males. We also compared the morphology of jack males with larger and older sockeye males and females to determine if jack males mimic females in their secondary sexual morphology or whether their morphology is more consistent to that of big males. Alternative morphologies, including female mimicry, have been linked to the existence of different strategies in fishes (e.g., Gross 1984) and other animals (e.g., Shuster 1992), whereas tactics are usually associated with rapidly adjustable behaviours and colours (e.g., Kodric-Brown 1986).

Methods

Body morphology

Body measurements were done on 43 mature males and 34 mature females collected below a beaver dam about 30 m upstream from the mouth of Pierre Creek, Babine Lake, in August 1989. Males were selected on the basis of size to obtain a roughly balanced sample from each of four predesignated fork length (FL) ranges (30–43 cm, 3 years old; 43–50 cm, 4 years old; 51–60 cm, 4–5 years old; 61–70 cm, 6 years old; Hanson and Smith 1967). The fish were killed in anaesthetic (MS 222) and the following measurements were made: (*i*) total weight (WT; ±1.0 g); (*ii*) gonad weight (GWT; ±1.0 g); (*iii*) fork length (FL; ±2 mm); (*iv*) postorbital hypural length (POH; ±2.0 mm; distance from the back of the orbit to the end of the hypural plate, this gives a length measurement unaffected by the development of secondary sexual characters); (*v*) maximum body depth (just forward of anterior insertion of dorsal fin (BD; ±1.0 mm); (*vi*) snout length (from the front of the orbit to the tip of the snout (SL; ±1.0 mm); (*vii*) skin thickness (ST; ±0.05 mm); (*viii*) maximum upper "canine" tooth length (TL; ±0.05 mm) and; (*ix*) adipose fin length (ADFIN; ±0.1 mm).

Skin thickness, including the dermis and epidermis, was measured with dial Vernier callipers from a sample of skin taken from a point one third of the vertical way from the lateral line to the insertion of the dorsal fin. Tooth measurements were done with callipers, fixing one end at the anterior base (gum) of the tooth and the other at the tip of the point of the tooth.

We analyzed the morphological variation by analysis of covariance of individual traits with body length (POH) and with principal components (PC) analysis of the data correlation matrix. Differences among groups in mean PC scores were compared with ANOVA followed by a posteriori Tukey's multiple comparison tests. Total "available sperm" volume (Liley et al. 1993) was measured for 45 males taken from spawning aggregations in nearby Fulton River, Babine Lake, proper or the associated spawning channel in September 1990. Available sperm volume was measured on fish killed by 2-phenoxyethanol. Each male was mounted upside down in a V-shaped holding trough, had its vent region cleaned and dried, and milt was expressed by gently stroking and squeezing its lower flanks. All emitted sperm was collected with an aspirator connected to a graduated cylinder (mL).

Spawning success of males

We measured the fertilization success of seven jack sockeye males (each less than 43 cm FL) and two kokanee males (each less 24 cm FL) sneaking in on spawning sockeye pairs (each >50 cm FL) in four 3.5 × 3.5 m pens placed end-to-end in a straight, wide (6 m) stretch of typical spawning habitat in Pierre Creek in August 1988.

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Fig. 1. Regressions of maximum body depth on postorbital hypural length for mature male (open large circle, solid line) and female (solid small circle, broken line) sockeye salmon from Pierre Creek, Babine Lake, British Columbia.

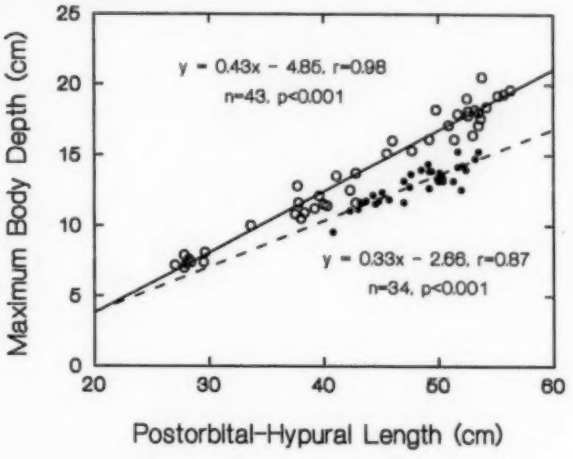
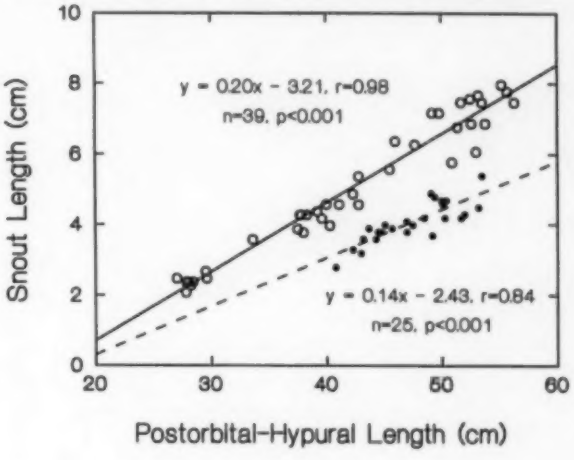


Fig. 2. Regressions of snout length on postorbital hypural length for mature male (open large circle) and female (solid small circle) sockeye salmon from Pierre Creek, Babine Lake, British Columbia.



The arenas were constructed of 2 cm mesh chicken wire. The methods were as follows. Where possible, we determined the parental genotypes from live fish at each of two loci (*PGM-2** and *ALAT**; see Wood et al. 1994 for assay methods). We anaesthetized the fish and removed a sample of muscle tissue (<0.5 cm^3) from the dorsal musculature using a surgical scalpel. The sample was immediately placed in a cooled sample tube and put on ice in a dark cooler and kept for electrophoretic analysis later that same evening. The incision was disinfected with antiseptic, the fish individually tagged (numbered Peterson disc tags for large males, and 2-cm colour coded Floy dart tags for jack and kokanee males) and allowed to recover in a 3.5 × 4.5 m holding pen. The wounds stayed free of infection, and the procedure appeared to have no effect on the behaviour of the fish. Initial tissue samples were not taken from four jack sockeye and two large sockeye (a male and a female) used near the end of the experiment; these fish were sampled after the experiment.

Where possible, we selected the combinations of fish to be used in the following day's trials based on their distinctive genotypes first by *ALAT** where three alleles are present in appreciable frequency in Babine sockeye, and second by *PGM-2** which has two alleles. Owing to the limited number of jacks available and the time it takes for a female to establish a nest site in an arena, we tested only seven individual jack males but often with multiple spawnings per female and across females. In the cases where we did not know the genotypes of the jack males, we sometimes switched the larger sockeye males between spawnings of a female to increase the probability of finding distinctive genetic markers by which to identify paternity (Appendix 1).

Once fish were selected, a minimum of two genetically acceptable females were placed in each arena on the evening before a trial and allowed to establish territories overnight (see Foote and Larkin 1988). In the morning, the female with the most excavated nest site was identified and the other female removed which increased the probability of selecting a female that would spawn in the near future. After 20 min, the large and jack sockeye males were added to the arena. Spawning behaviour observed by two to four people over the rest of the day. When spawning was observed, the participants, their positions immediately prior to spawning and their positions during spawning were recorded. The distance of the jack male from the female immediately prior to spawning (distance from the tip of jack's snout to the female's tail) was estimated (±10 cm) using the known length of the female for calibration). Immediately after spawning, two of us entered the arena, one placed a fine plastic meshed rectangular cage immediately behind the nest and the other excavated the nest by gently removing any stones and gravel until the egg mass was exposed. The eggs were brought out of the gravel by creating a strong upward current with both hands so that egg washed downstream into the cage. We attempted to collect all of the eggs in the nest. Few eggs appeared to suffer mortality from this method.

The eggs were placed in 1-L glass jars, filled to the brim with creek water, sealed and stored in the stream under a black tarpaulin until the end of the day. They were then packed in coolers and transported by boat (about 25 km) to the Fulton River Spawning Enhancement Facility, put into individually labelled baskets, placed in Heath trays and incubated on cooled Fulton River water (8–13°C) until the eyed egg stage (middle to late September). All dead eggs were then removed and the rest were transported by air to the Rosewall Creek Experimental hatchery on Vancouver Island where the eggs were held in about 8.5°C water until placed in rearing tanks in late November to early December. Due to time constraints, mortality during all stages was not recorded, but overall it appeared to be remarkably low (<10%), with no single cross experiencing significant mortality.

The fry from each spawning were reared in individual 400-L tanks for at least 4 months, after which approximately 100 fry were nonselectively collected from each tank by lowering the water to about 5 cm and sampling with a 15-cm dipnet, killed in MS 222, immediately frozen (–20°C), and then transported to a –40°C freezer. Each fish was then analyzed at the two predescribed loci. In addition, the genotypes of all parental fish were verified at this time from additional tissue samples taken from each parent at the end of the experiments.

Analyses of paternity results were done using the arcsine square-root transformed percentage estimates of progeny sired by each male.

Results

Morphology

When relationships between selected morphological variables and fish length were examined via ANCOVA we found that for a given size, males had greater maximum body depth, longer snouts, larger adipose fins, larger teeth, and thicker skin than females (Figs. 1–5). The slopes of various regressions of character size on body size did not differ significantly between

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Fig. 3. Regressions of adipose fin length on postorbital hypural length for mature male (open large circle) and female (solid small circle) sockeye salmon from Pierre Creek, Babine Lake, British Columbia.

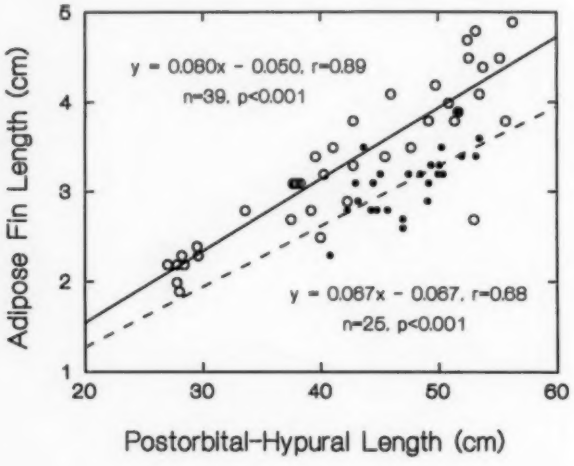
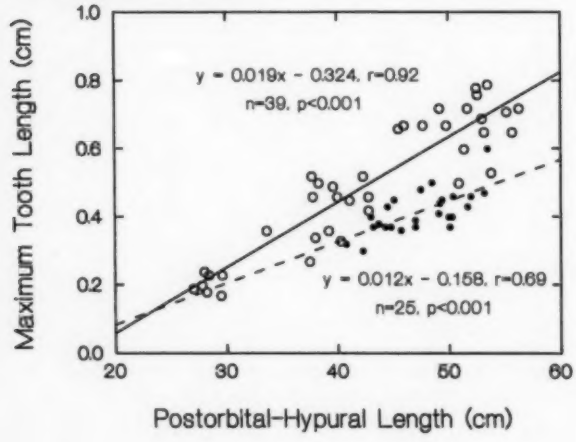


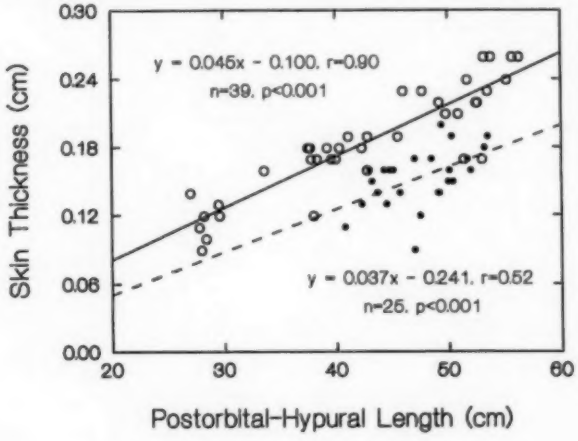
Fig. 4. Regressions of maximum upper jaw "canine" tooth length on postorbital hypural length for mature male (open large circle) and female (solid small circle) sockeye salmon from Pierre Creek, Babine Lake, British Columbia.



males and females ($P > 0.05$), but the slopes were always slightly greater for males than for females. The above relationships remained independent of whether jacks were included in the male group. That is, the inclusion of a male age-class outside of the range of all female sizes and that of older males, did not affect the pattern of the regression lines of males with respect to females. However, if jacks were included with the females (as it could be argued if they mimicked females in their morphology), then there were significant differences in the slopes of the regressions of body depth and snout length on body length ($P < 0.001$). We therefore conclude that jack morphology was more similar to that of larger males than females.

In the principal components analysis, PC1 accounted for

Fig. 5. Regressions of skin thickness on postorbital hypural length for mature male (open large circle) and female (solid small circle) sockeye salmon from Pierre Creek, Babine Lake, British Columbia.



88.03% of the variation, with size the principal loading factor (Pearson's r for ln fork length (LFL) and PC1 = 0.945; $n = 64$, $P < 0.001$). PC2 was only slightly correlated with LFL ($r = 0.301$; $P < 0.05$), and PC3 was uncorrelated with LFL ($r = 0.059$; $P > 0.5$; Table 1). When PC2 was broken into groups, only large males showed a marginally significant correlation with LFL ($r = 0.318$, $n = 31$, $P < 0.10$), with the correlation for females ($r = -0.014$, $n = 25$) and jacks ($r = 0.110$, $n = 8$) small and nonsignificant. Hence, PC2 and PC3 provide a largely size-independent comparison of body morphology on which to compare jacks with larger males and all females.

PC2 loaded most heavily on skin thickness and next on various aspects of size (ln POH (LPOH) and ln WT (LWT)) and sexually dimorphic traits (ln SL (LSL) and ln ADFIN (LADFIN)). PC3 loaded most heavily on the sexually dimorphic traits ln TL (LTL), LSL, and LADFIN. Scores derived from PC2 and PC3 differed significantly across groups ($P < 0.001$, ANOVA; Table 1). Jacks did not differ from large males on either PC score but differed significantly from females on PC2 ($P < 0.001$ Tukey's test). Large males differed from females on both PC scores ($P < 0.01$). Hence, jack males showed greater morphological affinity to large males than they did to females, with thicker skin, and longer adipose fins, snouts, and teeth than that expected of similar-sized females.

Gonad size and available sperm volumes

Male gonad size increased allometrically with length ($P < 0.001$), such that the relative proportion of the total body weight accounted for by gonad weight (gonadosomatic index) decreased with increasing length in males ($P < 0.001$; Fig. 6). Jack males had the largest gonads relative to their body size of all males (4.3% for jacks versus 2.1% for males over 525 mm). Similarly, available sperm volume was positively related to male POH ($P < 0.001$; Fig. 7). Available sperm volume varied from a minimum of 0.4 mL in a jack male to a maximum of 32.0 mL in a large male.

Spawning success of males

In total, 17 spawnings occurred involving a total of 10 females,

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Table 1. Character component loadings from a principal components analysis of morphological variation among large sockeye males, females, and jack sockeye males.

Character	PC1	PC2	PC3
Loadings			
LPOH	0.920	0.363	0.094
LWT	0.960	0.253	0.082
LBD	0.989	0.044	–0.001
LSL	0.966	0.253	0.082
LADFIN	0.934	–0.127	–0.187
LUT	0.941	0.012	–0.308
LSKIN	0.864	–0.455	0.098
% variance			
explained	88.304	6.224	2.493
Standardized scores			
Jack males	–1.986±0.052	–0.717±0.238	0.069±0.390
Large males	0.540±0.140	–0.603±0.103	–0.366±0.187
Females	–0.034±0.083	0.977±0.134	0.432±0.148

Note: The natural logarithms of the following characters were used in the analysis: postorbital hypural length (LPOH), total weight (LWT), maximum body depth (LBD), snout length (LSL), adipose fin length (LADFIN), maximum upper tooth length (LUT) and skin thickness (LSKIN). Values for standardized PC scores for jacks, large males, and females are mean ± SE.

11 large males, 7 jack males, and 2 kokanee males. The two kokanee males participated in a total of five spawnings, which also involved a jack and large sockeye male. One of the two jacks tested with the kokanee was observed not to participate in the first of two sockeye spawnings, whereas the kokanee participated in both events. Spawning events occurred in all pens, and there appeared to be no difference in the success of jack males among them. However, we could not accurately test this as some males were tested in more than one arena. When this confounding was ignored and all data were treated as independent, we found no significant difference among pens in jack spawning success ($P > 0.10$, ANOVA).

The large male was the closest to the female prior to spawning in all cases. All jacks and kokanee males assumed satellite positions downstream of the spawning sockeye pair, often hiding under tree limbs we placed in the pens, from where they acted as sneaks. In all 11 spawnings where jacks were observed to participate, they rushed in on the side of the female not occupied by the large male ($P < 0.001$, Sign test), even though this meant moving around the large male and female in some cases. In the five unobserved spawnings, the jack was situated directly downstream or lateral to the pair prior to the spawning event and was observed to have participated in other spawnings from the same position with the same pair on three of these occasions. Thus, we assumed the jack had participated in these spawnings as well.

We were able to directly estimate the contribution of the jack male to the overall paternity of the young in 15 of the 16 spawnings. In the case where we had no distinctive allele to mark the jack's contribution (Appendix 1, spawning 17), we were able to estimate the sockeye male's contribution through its own distinctive allele and, hence, indirectly estimate the jack's percentage contribution by subtraction. Only seven of the progeny sampled (2 of 1666 scored for *PGM-2** and 5 of the 1655 for *ALAT**) had genotypes inconsistent with the potential parents; these seven were ignored in subsequent analyses.

Fig. 6. Regression of gonadosomatic index (the proportion of gonad weight to total weight) on postorbital hypural length for mature sockeye males from Pierre Creek, Babine Lake, British Columbia.

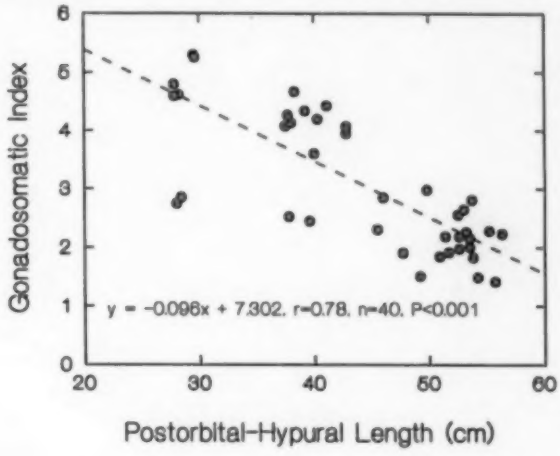
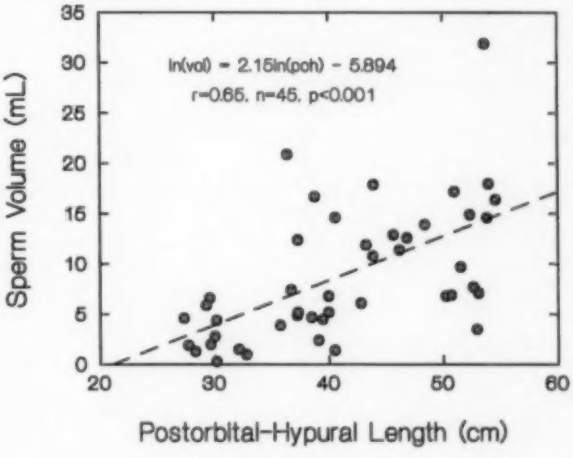


Fig. 7. Regression of available sperm volume on postorbital hypural length for mature sockeye males from Fulton River, Babine Lake, British Columbia.



In 11 cases involving 10 spawnings, we were able to derive two independent estimates of a male's spawning success through the presence of distinct alleles at both loci assayed in a parental fish or through the comparison of independent estimates derived through distinct alleles at separate loci of participating males. These could then be compared. The percent contribution of sneak males estimated at the *PGM-2** locus was strongly related to the contribution estimated at *ALAT** ($r = 0.951$; $P < 0.001$). All analyses of paternity results were therefore based on data averaged across loci where possible.

In the 12 spawnings where only jack and large sockeye males were present, jacks sired on average 42.2 ± 8.1% (mean ± SE) of progeny sampled, with a range of approximately 3–93%. The mean average success of these five jacks is higher (47.9 ± 11.8%), because one male spawned only once

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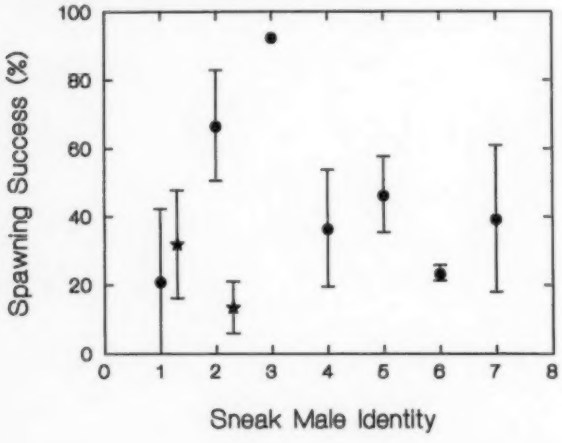
Fig. 8. Mean percent spawning success (±SE) for seven individual jack sockeye males (solid circles, 1–7) and two kokanee males (solid stars, participating with jacks 1 and 2) when in spawning competition with a larger, older sockeye male.



but fertilized 93% of the progeny sampled, and this single estimate raised the average for all jacks. When all 12 spawnings are treated as independent events (i.e., the least conservative test), we found no significant difference between the two male types (Wilcoxin paired signed ranks test, $P > 0.3$). Similarly, there was no significant difference in the mean success of the five jack and sockeye males tested ($P = 0.5$).

In the five spawnings where a jack male, a kokanee male and a large sockeye male were present, the kokanee male fertilized an estimated 20.9 ± 7.9% (range 0–47.8%) of the eggs laid, and the jack fertilized 48.5 ± 15.8% (range 0–87.2%; Fig. 8), even though in one case the jack male was observed not to participate (i.e., jack = 0). We observed the positions of the various males during three of these spawnings. In two independent cases, kokanee males relegated to a position on the side of the jack or sockeye male and, hence, at the greatest distance from the female, fertilized 14.9 and 16.2% of the eggs laid, respectively. In the other case, the kokanee disappeared beneath the spawning fish and fertilized 25.7% of the eggs.

We found no significant correlation between the ratio of the size of the jack to the large male to the jack's spawning success (both values averaged per jack) independent of whether we included or excluded the results of the two jacks which also competed with kokanee ($r = -0.535$, $n = 7$ and $r = -0.713$, $n = 5$, respectively).

We noted the distance of the jack male from the female immediately before spawning on nine occasions, involving five different jack males (Appendix 1). These distances were similar to those seen in Pierre Creek proper, where most sneak males are situated within 1 m of the tail of the female prior to spawning (Foote and Larkin 1988). This suggests the results in the pens are likely a good estimate of those free in the natural stream. Whether all data were treated as independent, or averaged for each male, there was no significant correlation of any type between distance from female prior to spawning and the proportion of progeny sired ($r_s = 0.413$ and 0.564, respectively; $P > 0.1$, Appendix 1). In the single case where the jack was observed to reach the female first (even though the large male was closer immediately prior to spawning), the large male fertilized an estimated 67% of the eggs. Similarly, in the one case we noted that the jack was physically closer to the female during spawning than the dominant male the jack only fertilized 3% of the eggs laid. In short, there is no clear relationship between distance and spawning success apparent in the data.

Discussion

Jack sockeye males contribute significantly to the fertilization of eggs when they sneak in on the older and larger spawning pairs. In this study they sired anywhere from 3 to 93% of the eggs fertilized. The consistent ability of males adopting a sneak tactic to fertilize at least a proportion of the eggs laid is consistent with other studies on salmonids (Schroder 1982; Chebanov et al. 1983; Maekawa and Onozato 1986; Hutchings and Myers 1988; Jordan and Youngson 1992).

The paternity results are unlikely to have resulted from sampling error, classification error, or differential mortality of the young. We collected nearly all of the eggs laid and subsequent mortality through to sampling was very low. In addition, we sampled about 100 fry per family so that the accuracy and precision of our paternity estimates is high. Finally, there was strong agreement in our paternity results in the two independent enzyme systems analyzed so there is little likelihood that classification error could account for the results.

On average, jack males fertilized 42% of the eggs laid when they participated in a spawning involving a single large male. This number was not significantly different from that of the large male, even though the jacks were kept at a position more distant from the female by the aggressive behavior of the large male and had smaller absolute gonad sizes and undoubtedly less available sperm per spawning act. However, jacks may have been able to mitigate some of these disadvantages by always moving in on the exposed female side, thus getting as physically close to the female during spawning as the large male. Further, they appeared able to position their vents closer to that of the female during spawning by sliding more under the belly of the female than the large males appeared able. Whether this behaviour improves their spawning success is unclear as in one case where we directly noted that the jack was closer throughout the spawning, it only fertilized 3% of the eggs laid.

Jacks may not have the opportunity to occupy the open side of a female in cases where there are more than one subdominant male, as is common in coho salmon (Gross 1985, 1991*a*). In that case, while the dominant male will undoubtedly occupy the position directly to one side of the female, the participant directly on the other side of the female will likely be decided by factors such as distance from that side of the female prior to spawning, the relative sizes of the males involved, and the speed at which the males can cover various distances. We observed that one kokanee sneak was closer to the female than the jack male prior to spawning, yet the jack was able to obtain the position directly beside the female, with the kokanee on the jack's other side. However, multiple subordinate males are not common in stream-spawning sockeye, at least during the main part of the run (e.g., Hanson and Smith 1967; Wood and Foote 1996), although multiple males do often occur when kokanee are present (McCart 1970; Foote and Larkin 1988).

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Both the size of the sneak male relative to the larger male paired with the female and the distance of the sneak male from the female prior to spawning have been related to the proportion of young sired in salmonids. Hutchings and Myers (1988) summarized the results of their own study on Atlantic salmon as well as those studies on other salmonids and observed that the average proportion of eggs fertilized by the smaller males was a positive function of the relative size of the small and large males. This observation is consistent with the prediction based on sperm competition that would be derived from the positive relationship between increased gonad size, available sperm volume, and body size of males observed in this study (see Moller 1991). Similarly, Schroder (1982) speculated that distance from a female before spawning was negatively correlated with the number of progeny sired in chum salmon. However, this observation is only generally supported by his data, which show that satellite chum salmon males fertilize about 30% of the eggs deposited during a spawning. No actual distance measurements were made, and thus it was impossible to assess how distance may have affected the gametic contribution of a satellite male. Nonetheless, this speculation has been crucial in later estimates of spawning success in salmonids where direct estimates of paternity were not available (e.g., Gross 1985; Fleming and Gross 1993).

Our data provide some support for Hutchings and Myers' (1988) observation. Kokanee males, which are smaller than jack males, sired a smaller proportion of the young than did jacks when they were paired together in this study, but our sample size was very small. (McCart (1970) was correct when he suggested that kokanee males successfully interbreed with sockeye females in Babine Lake tributaries.) Similarly, averaged over all spawnings, jacks sired fewer, but not significantly fewer, young than did the larger males. However, the results were extremely variable (even for individual males; Fig. 8, Appendix 1), as they were in Hutchings and Myers' (1988) compilation.

We found no clear evidence that distance from the female immediately prior to spawning was negatively related to the proportion of the young sired. Jack males fared nearly as well as the large sockeye males, even though they were always kept further from the female by the large male's and sometimes female's (see Maekawa et al. 1993) aggressive behavior. There was no suggestion of a negative relationship between spawning distance and fertilization success independent of whether we treated the data independently or averaged success and distance over each jack male. Our observations indicated that the jacks most often were not only fully aware of the onset of spawning but anticipated it such that they timed their arrival and release of sperm to coincide with the release of gametes by the sockeye pair.

Taken together, at least with jack and large sockeye males, we are not able to predict the relative spawning success of males even knowing the relative size of the males involved, their likely gonad size and available sperm volume, and their distance from the female immediately prior to spawning.

Our results suggest that jack sockeye are likely very successful on the spawning grounds. The question remains as to how such a system, with multiple male age-classes and alternative mating tactics, evolved. Numerous explanations have been proposed, ranging from frequency-dependent mixed evolutionarily stable strategies (Gross 1985), to a single mixed strategy involving two environmentally induced tactics (Bohlin et al. 1990; Repka and Gross 1995). In these cases, the two tactics may have equal or different finesses. Indeed, it is possible that it is the large males who are making the best of a bad situation, given that they experience the risk of mortality for an additional 1–3 years at sea before maturity compared with jack males (Thorpe 1986, 1987; Gross 1996). The high average spawning success of jack males shown here, and the fact that they are the faster growing and youngest males in the spawning population (Gross 1991*a*; C.C. Wood and C.J. Foote, unpublished data for Pierre Creek sockeye), adds credence to this hypothesis.

Whatever the case, the answer is unlikely to be simple. The mating behavior displayed by different-sized and -aged males is conditional; jack sockeye males switch courting tactics depending on the presence or absence of larger males (Foote and Larkin 1988). The mating success of each form is likely frequency dependent (Gross 1985) and density dependent, which complicates evolutionary interpretation of results (Eadie and Fryxell 1992). At high population densities, jack sockeye will likely be subject to harassment from all sides, independent of their frequency in the population. Production of jack males is conditional in salmonids; rapid growth promotes early maturity (e.g., Bilton et al. 1982; Thorpe 1987; Rowe and Thorpe 1990). Jack sockeye males do not have a distinct morphology, either externally (see Noakes et al. 1995) or internally (gonad size), which is consistent with the conditional nature of the different life-history tactics. However, there is also an additive genetic component to age at maturity in Pacific salmon (Silverstein and Hershberger 1992; Hankin et al. 1993; Heath et al. 1994; C.C. Wood and C.J. Foote, unpublished data for Pierre Creek sockeye), although it is not a simple Mendelian trait producing discrete strategies (see Gross 1996). The combination of genetic and environmental factors in the production of various male age-classes at maturity, the conditional nature of spawning tactics employed, and the frequency- and density-dependent effects on fitness of each type makes it very difficult to estimate and compare the fitness of various age-classes of males, let alone test among competing models for their evolution.

We believe the most likely answer lies in models that combine condition dependent choice of life- history tactics with factors that account for genetic variation in the threshold for the tactic expression within populations. The elegant model of Bohlin et al. (1990; see also Repka and Gross 1995) for within population variation in life-history tactics, combined with their extension to account for genetic variation among populations, may be appropriate to explain the genetic and environmental variation in life-history tactics observed within Pacific salmon populations.

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Appendix 1

Summary of parental data (fork length and genotype at *ALAT** and *PGM-2**), genotype frequencies of progeny, estimators of parental components (i.e., genotypic identity) for jack (J), kokanee (K), and large sockeye males (L), estimates of % paternity at each loci, average paternity and distance from female tail of each sneak male prior to spawning for each of 17 spawning events (SP) with 10 sockeye females (S).

													Genotypes of progeny[b]							
	Fish identities, fork lengths, and genotypes												*ALAT**					*PGM-2**		
SP	L♂	FL[a]	*ALAT**	*PGM–2**	S♀	FL	*ALAT**	*PGM–2**	J♂/K♂	FL	*ALAT**	*PGM–2**	AA	AB	AC	BB	BC	AA	AB	BB
1	1	624	AA	AA	1	533	AA	AA	1	343	AC	AB	85	0	16	0	0	81	19	0
									1	233_K	BC	AA								
2	1	624	AA	AA	1	533	AA	AA	1	343	AC	AB	40	13	48	0	0	57	44	0
									1	233_K	BC	AA								
3	1	624	AA	AA	1	533	AA	AA	1	343	AC	AB	41	7	46	0	0	51	43	0
									1	233_K	BC	AA								
4	2	642	AA	AA	2	640	AA	BB	2	324	BB	AA	42	59	0	0	0	0	79	18
									2	218_K	BB	AB								
5	2	642	AA	AA	2	640	AA	BB	2	324	BB	AA	42	59	0	0	0	91	8	0
									2	218_K	BB	AB								
6	3	611	BB	AA	3	538	AC	AB	3	336	AA	BB	58	2	53	0	1	2	59	48
7	4	565	AA	AA	4	516	AB	AB	4	357	AB	AA	34	54	0	12	0	54	46	0
8	5	560	AA	AA	4	516	AB	AB	4	357	AB	AA	47	49	0	4	0	52	47	1_7
9	6	605	BB	AA	5	546	BB	AB	4	357	AB	AA	0	39	0	49	0	44	54	0
10	7	524	BB	AB	6	565	AB	BB	4	357	AB	AA	2	47	0	51	0	0	49	51
11	5	560	AA	AA	7	534	BB	AB	5	349	AB	AB	1_7	82	0	17	0	37	55	8
12	8	588	BB	AA	8	544	BB	AA	5	349	AB	AB	4_7	19	0	76	0	81	19	0
13	8	588	BB	AA	8	544	BB	AA	5	349	AB	AB	0	32	0	66	0	64	36	0
14	5	560	AA	AA	7	534	BB	AB	6	373	AB	AA	0	86	0	13	0	49	50	0
15	5	560	AA	AA	7	534	BB	AB	6	373	AB	AA	0	69	0	8	0	43	34	0
16	10	578	AB	AA	9	546	BB	AB	7	426	AA	AB	0	58	0	42	0	53	42	5
17	11	565	AB	AB	10	535	AB	AA	7	426	AA	AB	36	38	0	8	0	46	35	1_7

Note: The subscripts are as follows: J, K, L, jack, kokanee, and large sockeye males, respectively; 1, jack *ALAT** contribution estimated through subtraction of known kokanee contribution (B allele) from known kokanee–jack combination (C allele); 2, jack observed not to participate in this spawning; 3, jack contribution at *ALAT** estimated by subtracting the independent estimate of kokanee contribution (*PGM-2**) from combined estimate of kokanee and jack contribution at *ALAT**; 4, jack and large sockeye male estimated contributions at *ALAT** are both the average of the results from two marker alleles; 5, jack contribution estimated solely by subtraction of estimated large sockeye male contribution from 100; 6, average percent jack paternity calculated by $(J + (100 - L))/2$; 7, genotypes of progeny not accounted for by possible parents are ignored in subsequent calculations of parental contributions.

[a]Fork length (FL) is in millimetres.

[b]Genotype designations are given by combinations of three alleles for *ALAT** and two alleles for *PGM-2**. For simplicity, they are designated by the letters A, B, and (for *ALAT**) C, where for *ALAT**, A = **100*, B = **91*, and C = **95* and for *PGM-2*, A = **100* and B = **135* (see Wood et al. 1994).

[c]Distance is in centimetres.

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Genotypic identity		% Progeny sired by				Distance from ♀	Average % paternity	
		J♂/K♂		L♂				
*ALAT**	*PGM-2**	*ALAT**	*PMG-2**	*ALAT**	*PGM-2**		J♂/K♂	L♂
50_K	50_J	31.7_J	38.0	—	—	—	34.8	—
		0.0_K	—				0.0_K	—
50_K	50_J	69.3_J	87.1	—	—	—	78.2	
		25.7_K	—			—	25.7_K	
50_K	50_J	83.0_J	91.5	—	—	—	87.2	—
		14.9_K	—				14.9_K	
100_{K2}	50_K	0.0	0.0	—	—	—	0.0	—
		58.4_K	37.1_K				47.8_K	
100_{JK}	50_K	42.3_J	—	—	—	—	42.3	—
		—	16.2_K				16.2_K	
100_{JL}	50_{JL}	97.4_4	88.1	2.6_4	3.7	70	92.7	3.2
25_J	0	48.0	—	—	—	25	48.0	—
25_J	0	16.0	—	—	—	—	16.0	—
50_J	0	79.6	—	—	—	50	79.6	—
25_J	50_L	8.0	—	—	102.0	50	3.0_6	102.0
50_J	25_J	32.0	34.0	—	—	40	33.0	—
50_J	50_J	40.0	38.0	—	—	50	39.0	—
50_J	50_J	65.3	72.0	—	—	50	68.7	—
50_J	0	26.3	—	—	—	50	26.3	—
50_J	0	20.8	—	—	—	—	20.8	—
50_L	25_J	—	20.0	84.0	—	50	18.0_6	84.0
25_L	0	61.0_5	—	39.0	—	—	61.0_5	39.0

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Latitudinal variation in vertebral number has a genetic basis in the Atlantic silverside, *Menidia menidia*[1]

Jean M. Billerbeck, Guillermo Ortí, and David O. Conover

Abstract: Variation in the number of vertebrae was examined in field and laboratory populations of the Atlantic silverside, *Menidia menidia*, drawn from across the species' range along the Atlantic coast of North America. Vertebral counts of wild fish increased and were tightly correlated ($r = 0.995$) with latitude in accordance with Jordan's rule. This strong correlation persisted in laboratory stocks reared for multiple generations in a range of common environments, revealing a genetic basis to Jordan's rule. Selection over the winter in the field increased mean vertebral number in the northern population, intensifying the latitudinal trend in vertebral number that existed in the fall. The combined field and laboratory data support the hypothesis that vertebral number in fishes is adaptive. Moreover, these data implicate natural selection as opposed to random genetic drift as the cause of Jordan's rule.

Résumé : La variation du nombre de vertèbres dans des populations naturelles et de laboratoire de capucettes, *Menidia menidia*, capturées dans l'ensemble de l'aire de répartition de l'espèce le long de la côte Atlantique de l'Amérique du Nord, a été examinée. Le nombre de vertèbres chez les poissons sauvages a augmenté en étroite corrélation ($r = 0{,}995$) avec la latitude conformément à la règle de Jordan. Cette forte corrélation s'est maintenue dans les stocks de laboratoire élevés pendant de nombreuses générations dans une variété d'environnements répandus, ce qui établirait un fondement génétique à la règle de Jordan. La sélection pendant l'hiver dans le milieu naturel a augmenté le nombre moyen de vertèbres dans la population septentrionale, accentuant la tendance liée à la latitude touchant le nombre de vertèbres qui existait à l'automne. Les données de terrain et de laboratoire combinées viennent appuyer l'hypothèse que le nombre de vertèbres observé chez les poissons serait un phénomène adaptatif. De plus, ces données viennent appuyer l'idée que la sélection naturelle, plutôt que la dérive génétique aléatoire, serait à l'origine de la règle de Jordan.
[Traduit par la Rédaction]

Introduction

The number of vertebrae in fishes varies geographically among both populations and species. Much of this variation can be attributed to Jordan's rule, the tendency for fish from higher latitudes and colder waters to have greater numbers of vertebrae than related fish from lower latitudes and warmer waters (Jordan 1891). This correlation between vertebral number and latitude occurs in many fish groups, at various taxonomic levels, in both the Northern and Southern hemispheres (Lindsey 1988). Although Jordan's rule is a widespread phenomenon discovered over a century ago, its cause is still unknown.

The ubiquity of Jordan's rule suggests that vertebral number is adaptive. Adaptive explanations for Jordan's rule involving latitudinal variation in selection on larval fishes have been suggested (Lindsey 1975; Swain 1988, 1992*a*). However, the genetic basis of phenotypic trends in nature must be shown before natural selection can be invoked (Endler 1986). Phenotypic variation in the wild may not accurately reflect genotypic variation because its origin may be genetic, environmental, or some combination of the two. Moreover, environmental and genetic influences can be positively (cogradient) or negatively (countergradient) correlated; thus, their net effect will determine the magnitude of phenotypic variation observed (Conover and Schultz 1995).

The extent to which Jordan's rule reflects genotypic or phenotypic differences among populations is currently unclear. Although the number of vertebrae in fishes is set at or near hatching and thereafter remains unchanged (Lindsey 1975), it is highly malleable during early development in response to environmental factors including temperature, light, salinity, and oxygen concentration (reviewed in Lindsey 1988). Additionally, the environment prior to fertilization has been shown to influence vertebral number (Dentry and Lindsey 1978; Swain and Lindsey 1986). Temperature is typically considered the operational factor in Jordan's rule, as the relationship between vertebral number and rearing temperature is negative in most cases (Lindsey 1988). Although many studies have explored the phenotypic plasticity of vertebral number in a given stock of fish, genetic differences among stocks have received little attention (Barlow 1961).

Common garden experiments provide one of the best means of studying genetic differences among populations across varied environments (Conover and Schultz 1995). Rearing populations for multiple generations over a range of conditions

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J.M. Billerbeck[2] **and G. Ortí.** Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, U.S.A.
D.O. Conover. Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794, U.S.A.

[1] Contribution No. 1071 of the Marine Sciences Research Center of the State University of New York at Stony Brook.
[2] Author to whom all correspondence should be addressed. e-mail:jeanm@life.bio.sunysb.edu

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enables the confounding effects of covariation between genotypes and the environment, as well as genotype-by-environment interactions, to be overcome. Only one common garden study of Jordan's rule has been attempted; laboratory rearing of chinook salmon, *Oncorhynchus tshawytscha*, from the Pacific coast of North America indicated a genetic basis to Jordan's rule (Seymour 1959). However, parental stocks were drawn from the wild in this experiment, leaving open the possibility of prefertilization environmental influences. Additionally, these findings in salmon may not represent the general case in fishes. Salmon are anadromous, homing fishes that may be more likely to show local adaptation than marine fishes with more panmictic populations (Carvalho 1993; Taylor 1991). Thus, the genetic basis of Jordan's rule has not been clearly demonstrated.

Here we examine variation in vertebral counts of the Atlantic silverside, *Menidia menidia*, a common marine and estuarine fish species. First, we present data from six sites across the geographic range of *M. menidia* to test for a latitudinal cline in vertebral number. Second, we compare the pattern of variation in wild fish from three of these sites to that of their descendants, reared for many generations in common laboratory environments, thereby assessing the role of genetics in Jordan's rule. Third, we compare field samples taken in the fall with those taken in the spring to determine if selection on vertebral number occurs over the winter, a period of high mortality in some populations of *M. menidia* (Conover 1984).

Materials and methods

Study organism

The Atlantic silverside, *M. menidia*, is a small, schooling fish of the family Atherinidae. It is one of the most abundant fishes in the shore zone (Bayliff 1950; Conover and Ross 1982) along the east coast of North America from northern Florida, U.S.A. (≈30° N) to Newfoundland, Canada (≈46° N) (Johnson 1975). *Menidia menidia* is essentially an annual species, completing its life cycle in 1 year (Conover and Ross 1982). Spawning occurs progressively later in the year at higher latitudes, resulting in a similar range of temperatures (12–30°C) during spawning across latitudes (Conover and Present 1990). In populations at higher latitudes, spawning occurs in June and July, and the growing season lasts until October (Conover and Present 1990) when individuals migrate offshore for the winter (Conover and Murawski 1982). Winter mortality in these northern populations is size dependent, and may be severe (Conover 1984). In populations at lower latitudes, spawning occurs from March to July, and the growing season extends into December (Conover and Present 1990). Unlike northern populations, the southernmost populations remain in the shore zone throughout the year (Conover and Murawski 1982).

Sampling procedures

Vertebral counts of fish from six locations along the east coast of North America were examined to determine the pattern of variation in the wild: Edisto Island, South Carolina (SC), ≈33°N; Beaufort, North Carolina (NC), ≈35°N; Upshurs Bay, Virginia (VA), ≈38°N; Great South Bay, New York (NY), ≈41°N; Annapolis Basin, Nova Scotia (NS), ≈44°N; and Prince Edward Island (PEI), ≈46°N. Embryos were collected in the field and hatched in the laboratory at 21°C as part of a study of environmental sex determination. Detailed procedures may be found in Conover and Heins (1987*a*).

Field specimens of juvenile and adult *M. menidia* were collected in the fall of 1988 and the spring of 1989 to examine changes in vertebral number over the winter. Fish were collected from shore at three of the above field sites (NS, NY, and SC) using a 30-m beach seine. Collection dates in the fall of 1988 corresponded roughly with the end of the growing season at the various latitudes: September 26 and 27 in NS, October 25 in NY, and November 28 in SC. Field sites were sampled in the spring of 1989 just prior to the onset of the spawning season: June 3 and 4 in NS, April 18 in NY, and March 20 in SC.

Laboratory specimens were drawn from stocks initiated with fish from three of the above field sites (NS, NY, and SC) and maintained at the Flax Pond Marine Laboratory in New York. The stocks were reared in a common environment (identical food, light cycles, water temperatures) for seven generations prior to sampling. Population size at each generation was maintained at several hundred (200–400) fish. Maturation and spawning of fish was induced through manipulation of the photoperiod, enabling the production of approximately two generations per year. Laboratory fish were hatched and reared at two temperatures within the range experienced by *Menidia* in nature during the spawning and growing seasons, 17 and 28°C. These temperatures represented the low and high temperature treatment groups of the sex determination study (Conover et al. 1992).

Vertebral analysis

Radiographs of fish were taken using a Hewlett-Packard Faxitron Series 43806 X-ray system (50 kVP, 6 mA, 60 s) and Kodak Industrex type M film. Numbers of vertebrae were scored from the radiographs using a dissecting scope with transmitted light. Vertebral counts included the centra between the basioccipital and the urostyle. Fish with equivocal counts (1.1%) due to vertebral abnormalities were excluded from the analyses. Additionally, sex and standard length of fish (to the nearest mm) were recorded when possible. Differences in vertebral counts between populations were tested via chi-square (χ^2) analysis. Analysis of covariance (ANCOVA) was performed when vertebral number and fish length were correlated.

Results

Field samples

The correlation between mean vertebral number and latitude was positive and highly significant ($r = 0.995$, $P < 0.0001$) among the six field locations sampled (Fig. 1). Similarly, vertebral numbers differed significantly (χ^2, $P < 0.0001$) among the three populations sampled in 1988 and 1989 (Fig. 2). This difference was particularly striking between the NS and SC populations, with virtually no overlap in their vertebral counts. Differences in vertebral number among populations could not be attributed to differences in fish length. Although fish standard length had a significant effect on vertebral count within populations (ANOVA, $P < 0.0001$ in each), differences between populations remained highly significant after correction for length (ANCOVA, $P < 0.0001$ in both years). Mean vertebral number increased significantly (χ^2, $P < 0.01$) over the winter in fish from NS (Fig. 2). Changes in vertebral number between the fall and spring samples were not significant in NY (χ^2, $P > 0.1$) or SC (χ^2, $P > 0.5$) fish. Mean standard length of fish in all field populations increased significantly (unequal variance t test, $P < 0.0001$ in each) over the winter (Fig. 3). Variance in fish length decreased over the winter in NS (F_{max}, $P < 0.0001$) and NY (F_{max}, $P < 0.01$) but increased in SC (F_{max}, $P < 0.001$). The increase in vertebral number over the winter in NS fish became nonsignificant when samples were corrected for size differences (ANCOVA, $P > 0.8$).

Sexual dimorphism in both vertebral number and size was evident in the SC field sample of 1989. Female fish had higher vertebral counts (χ^2, $P < 0.05$) and were larger than males

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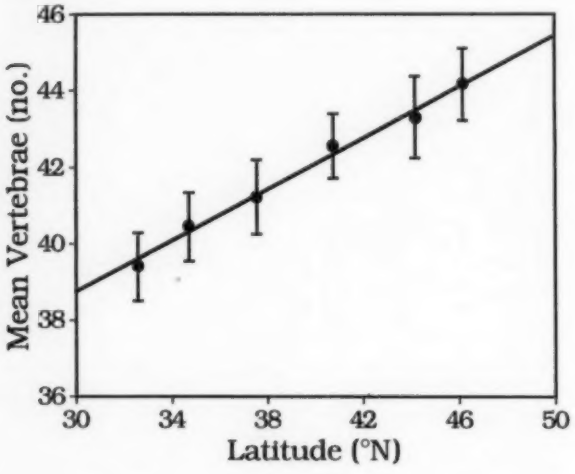
Fig. 1. Correlation between latitude and mean number of vertebrae (±1 SD) among populations of *M. menidia* along the east coast of North America ($r = 0.995$, $P < 0.0001$, $n = 6$). Sample sizes at the various latitudes were SC = 55, NC = 87, VA = 48, NY = 41, NS = 32, and PEI = 49.



(t test, $P < 0.0001$). Sexual dimorphism in size occurred in SC in 1988 (t test, $P < 0.0001$) and in NY in 1988 (t test, $P < 0.05$) and 1989 (t test, $P < 0.0001$), with females larger than males. Sexual dimorphism in vertebral number was evident in NS in 1989 (χ^2, $P < 0.01$), with males having greater numbers of vertebrae than females. Correction for size rendered sex differences in vertebral number nonsignificant (ANCOVA, $P > 0.1$ in NY and $P > 0.9$ in SC).

Laboratory samples

Vertebral numbers differed significantly (χ^2, $P < 0.0001$) among laboratory-reared stocks at both experimental temperatures: NS fish had the highest counts, NY fish had intermediate counts, and SC had the lowest counts (Fig. 4). Similar to the pattern in the field, vertebral counts of NS and SC fish raised in common environments were nonoverlapping. The relationship between vertebral number and rearing temperature was negative. Vertebral numbers at 17°C were significantly greater than those at 28°C across all laboratory stocks (χ^2, $P < 0.001$). Neither fish length (ANOVA, $P > 0.05$) nor fish gender (χ^2, $P > 0.05$) had a significant effect on vertebral number in the laboratory populations.

Discussion

The positive correlation between vertebral number and latitude in *M. menidia* drawn from the wild conforms with Jordan's rule. The results of our common garden experiments clearly demonstrate the genetic basis of Jordan's rule in *M. menidia*; differences in vertebral number among populations from different latitudes persisted over multiple generations in common laboratory environments. These combined field and laboratory data point to natural selection rather than random genetic drift as the cause of Jordan's rule. Some phenotypic plasticity of vertebral number in response to temperature was evident; low developmental temperature resulted in slightly higher vertebral counts. However, no genotype-by-temperature interaction was observed. The reaction norms of vertebral counts in response to temperature were similar across populations within the temperature range experienced by *M. menidia* during the spawning and growing seasons. This phenotypic plasticity may slightly enhance the magnitude of variation in vertebral counts across latitudes in the wild. Thus, environmental and genetic influences on vertebral number in *M. menidia* are cogradient.

The genetic basis of the latitudinal cline in vertebral counts in *M. menidia* supports the hypothesis that vertebral number in fishes is adaptive, yet the selective factors remain unclear.

Fig. 2. Frequency distributions of vertebral counts of *M. menidia* from Nova Scotia, Canada (NS), New York, U.S.A. (NY), and South Carolina, U.S.A. (SC), sampled in fall 1988 and spring 1989. Numbers of vertebrae differed significantly between populations in each year (χ^2, $P < 0.0001$). Mean vertebral number increased significantly in NS over the winter (χ^2, $P < 0.01$), steepening the cline that existed in the fall. Means ± SE and sample sizes in fall 1988 were 43.7 ± 0.1, $n = 95$ in NS; 43.0 ± 0.1, $n = 125$ in NY; and 39.2 ± 0.1, $n = 105$ in SC. These statistics in spring 1989 were 44.1 ± 0.1, $n = 145$ in NS; 43.2 ± 0.1, $n = 151$ in NY; and 39.0 ± 0.1, $n = 93$ in SC.

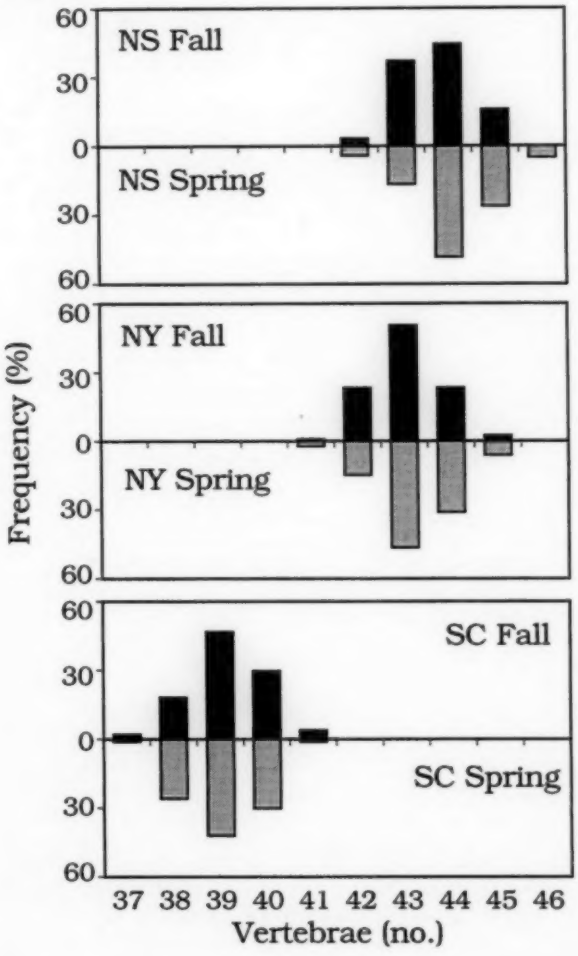


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Fig. 3. Frequency distributions of standard lengths of field populations from NS, NY, and SC. Size-dependent winter mortality in NS and NY is evidenced by changes in the length–frequency distributions from fall to spring. Means ± SE in fall 1988 were 62.3 ± 1.2 in NS, 64.5 ± 1.0 in NY, and 65.2 ± 0.5 in SC. These statistics in spring 1989 were 78.8 ± 0.5 in NS, 82.5 ± 0.7 in NY, and 71.2 ± 0.8 in SC.

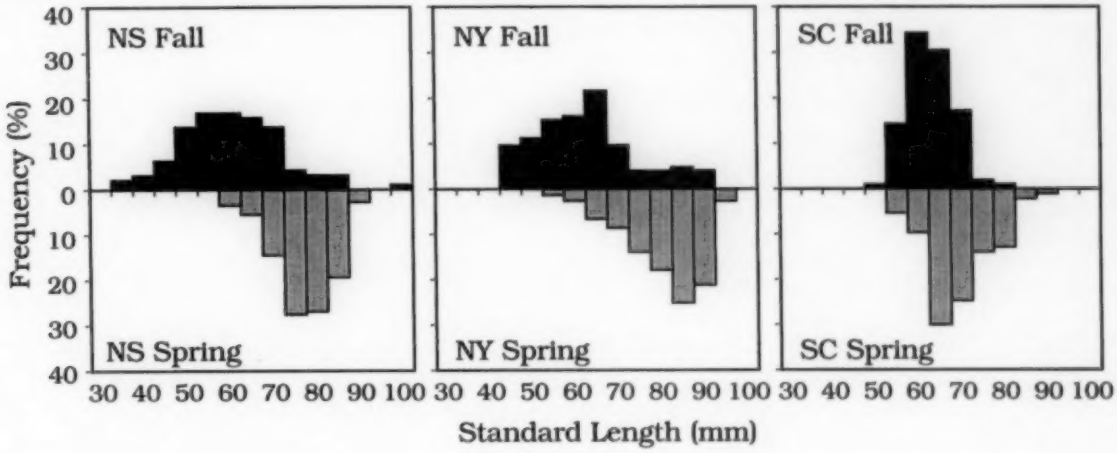
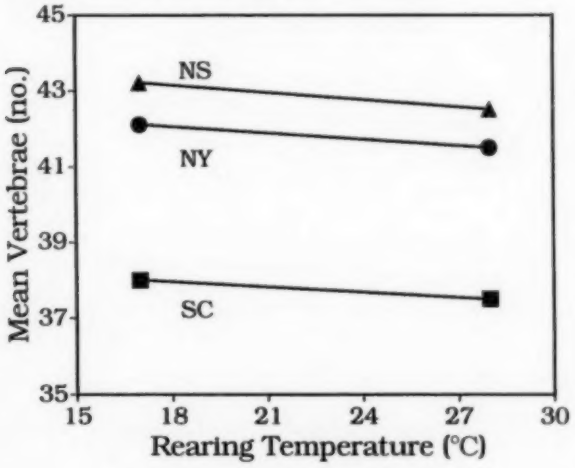


Fig. 4. Reaction norm of vertebral number in response to developmental temperature. Means ± SE and sample sizes for the 17°C treatment were 43.2 ± 0.1, $n = 86$ in NS; 42.1 ± 0.1, $n = 43$ in NY; and 38.0 ± 0.2, $n = 45$ in SC. These statistics for the 28°C treatment were 42.5 ± 0.1, $n = 126$ in NS; 41.5 ± 0.1, $n = 134$ in NY; and 37.5 ± 0.1, $n = 127$ in SC.



A functional relationship between vertebral number and swimming performance has been theorized (Lindsey 1975; Spouge and Larkin 1979). Direct evidence of the adaptive nature of vertebral counts has come from experiments demonstrating selective predation for vertebral number in larval sticklebacks, *Gasterosteus aculeatus* (Swain and Lindsey 1984), and peamouth, *Mylocheilus caurinus* (Swain 1988). Recently, this selective predation has been linked to differences in burst swimming speeds of different vertebral phenotypes (Swain 1992*a*, 1992*b*).

Hydrodynamic theory suggests that the vertebral phenotype conferring optimal swimming performance in fishes may depend upon temperature and fish shape (Spouge and Larkin 1979). The viscosity of seawater changes with temperature, approximately doubling between tropical and arctic waters. The hydrodynamic consequences of this may result in selection for different vertebral numbers across latitudes. However, selective predation on vertebral number has not been shown to vary with temperature, even across temperature ranges as great as 10°C (Swain and Lindsey 1984; Swain 1988). The model of Spouge and Larkin (1979) also predicts that more rotund fish should have fewer vertebrae. In at least four families of fish, vertebral number and body depth are negatively correlated (Lindsey 1988). Southern populations of *M. menidia* are deeper bodied than northern populations (Kendall 1902; Morgan and Ulanowicz 1976). Thus, differences in body depth among *M. menidia* populations may affect the cline in vertebral number.

The optimal vertebral phenotype may also depend upon fish size (Spouge and Larkin 1979). Lindsey (1975) noted a positive correlation between maximum adult size and vertebral number (pleomerism) among many fish groups. Based upon these observations, he suggested that Jordan's rule might be explained by latitudinal differences in survivorship curves resulting in the matching of vertebral number to different critical body sizes across latitudes. Although pleomerism was described in terms of adult body size, Lindsey (1975) suggested that the strongest selection on vertebral number in fishes probably occurs in the early larval stages. Predation experiments have demonstrated selection on vertebral number within a narrow range of larval sizes (Swain 1988, 1992*a*). Differential selection on vertebral phenotypes across latitudes may occur in larval *Menidia*. Larval growth rates in *M. menidia* are genetically based and positively correlated with latitude (Conover and Present 1990). However, silversides hatch at similar sizes across latitudes, and no subsequent latitudinal trends in larval sizes are known.

Selection on vertebral number in juvenile and adult fish in the first winter of life may play an important role in Jordan's rule. Conover and Ross (1982) reported winter mortality rates of *M. menidia* as high as 90–99%. Selection over the winter may therefore be as strong as that in the early larval stages.

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Overwinter mortality has been suggested as the cause of genetic variation across latitudes in a suite of life history traits in *M. menidia*. These traits include differences in food consumption and growth rates (Conover and Present 1990), growth efficiency (Present and Conover 1992), initiation and duration of spawning (Conover and Present 1990), sex determination (Conover and Heins 1987*a*), and sex ratio (Conover and Heins 1987*b*). Size- and vertebral-selective mortality of *M. menidia* in the northern populations is indicated by changes in the distributions of these characters over the winter. The increase in the mean number of vertebrae in the northern population intensified the latitudinal trend in vertebral number that existed in the fall. Size-dependent winter mortality, as evidenced by increases in mean length accompanied by decreases in the variance in length, has been reported previously in northern populations of *M. menidia* (Conover 1984; Conover and Present 1990). Contrary to the situation at higher latitudes, the increase in both mean and variance in length in the southern population is more consistent with growth rather than selective overwinter mortality.

The relationship between size and vertebral number in *M. menidia* is difficult to interpret from our study. Selection for vertebral number over the winter cannot be distinguished from selection for size, because fish length and vertebral number were correlated within field populations. Pleomerism among populations in the field, however, did not exist. Similarly, fish length and vertebral number were not correlated within or among laboratory populations. The correlations between length and vertebral counts within wild populations could reflect either genetic differences between early and late spawning fish or selection on vertebral number during the growing season. However, temperature changes over the spawning season more likely were the cause. Larger fish were probably spawned earlier in the season at colder temperatures, resulting in higher vertebral counts. The tendency for larger fish of a year-class to have more vertebrae than smaller fish of the same year-class has been shown in other spring-spawning species (Ben-Tuvia 1963; Komada 1982).

Pleomerism was also evident between sexes in *M. menidia* in the southern population; females were larger than males and had more vertebrae. Pleomerism between sexes within a population is rare but has been shown to occur in three congeneric species of blennies and in one shark species (Lindsey 1975). This sexual dimorphism in size and vertebral number corresponds to known patterns of sex determination in silversides (Conover and Heins 1987*a*, 1987*b*). Environmental sex determination (ESD) in *M. menidia* causes fish spawned earlier in the season at colder temperatures to become female. The strength of ESD is correlated with latitude in *M. menidia*, with the highest level of ESD in the south. Therefore, pleomerism between the sexes in fish from South Carolina is most likely due to the combined effects of ESD and vertebral plasticity in response to temperature.

In summary, our study provides the first demonstration that Jordan's rule has a genetic basis. Moreover, the close correspondence between latitude and vertebral number in *M. menidia* implicates natural selection as opposed to random genetic drift as the cause of Jordan's rule. Although most adaptive explanations of Jordan's rule focus on selection in larval fishes, we provide evidence that selection on adult *Menidia* over the winter may also play an important role in the variation of vertebral number with latitude. Further analyses and experiments are necessary to fully elucidate the selective agents responsible for Jordan's rule.

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Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities?

Michelle F. Bowman and Robert C. Bailey

Abstract: Ten data sets from published studies of freshwater benthic macroinvertebrates were used to deduce the necessary level of taxonomic resolution (from genus to phylum) for assessing variation in community structure. Quantitative data sets describing communities of benthic invertebrates identified to genus were aggregated to family, order, class, and phylum. Bray–Curtis distances between each pair of communities in a given study were calculated at each level of taxonomic aggregation. Also, quantitative data were converted to qualitative (presence–absence) data and aggregated in the same manner. Jaccard's distances between communities were calculated for these data matrices. Correlations between the resulting distance matrices, calculated at different taxonomic resolutions, were quantified using Mantel's test (N. Mantel. 1967. Cancer Res. **27**: 209–220). Distance matrices for different taxonomic resolutions were highly correlated ($r >> 0.64$) when based on quantitative data for all except 1 of the 10 studies. Correlations were lower and more variable for qualitative data sets. For the data sets investigated, genus-level identification did not usually provide a strikingly different description of community patterns than higher levels (e.g., family, order) of taxonomic identification.

Résumé : Dix ensembles de données tirés d'études publiées sur les macroinvertébrés benthiques d'eau douce ont été utilisés pour déduire le degré nécessaire de résolution taxinomique (allant du genre au phylum) pour évaluer la variation de la structure de la communauté. Des ensembles de données quantitatives décrivant les communautés d'invertébrés benthiques identifiés jusqu'au niveau du genre ont été regroupés par famille, ordre, classe et phylum. Les distances de Bray–Curtis entre chaque paire de communauté dans une étude donnée ont été calculées à chaque niveau d'agrégation taxinomique. De plus, les données quantitatives ont été converties en données qualitatives (présence–absence) et regroupées de la même manière. Les distances de Jaccard entre les communautés ont été calculées pour ces matrices de données. Les corrélations entre les matrices de distances résultantes, calculées à différents degrés de résolution taxinomique, ont été quantifiées à l'aide du test de Mantel (N. Mantel. 1967. Cancer Res. **27**: 209–220). Les matrices de distance pour différentes résolutions taxinomiques présentaient une forte corrélation ($r >> 0{,}64$) lorsqu'elles étaient fondées sur des données quantitatives pour 9 des 10 études. Les corrélations étaient plus faibles et plus variables pour les ensembles de données qualitatives. Dans le cas des ensembles de données étudiés, l'identification jusqu'au niveau du genre n'a pas produit, de manière générale, une description substantiellement différente des caractéristiques de la communauté que l'identification taxinomique à des niveaux plus élevés (p. ex. famille, ordre).
[Traduit par la Rédaction]

Introduction

A community may be defined as a set of species co-occurring in space and time, and community structure as the variation and covariation of the species across some spatial or temporal scale (Bailey 1992). Analysis of variation in community structure is clearly a multivariate problem, with the taxa in a community being the "response variables" of interest. A multivariate consideration of community structure results in a distance matrix of pairwise differences between communities. Structure is then described by subjecting the distance matrix to ordination or cluster analysis. Research or assessment requiring an adequate description of such variation, and particularly detection of changes due to human impact, is almost always a compromise between the scientific ideal and political, financial, and logistic limitations (Warwick 1993). One important constraint is the amount of resources available for identifying invertebrates in the communities sampled.

Identification of organisms in the community to a taxonomic level adequate to meet the objectives of a study has been called "taxonomic sufficiency" (Ellis 1985). Identification to the species level is not always feasible because of limited taxonomic expertise, time, and (or) money, and biological features to identify the organisms collected at a given place and time (Furse et al. 1984). The multivariate statistical techniques often used to describe variation in community structure are often sensitive to the number of variables (i.e., taxa) used to describe each observation (i.e., community). Researchers face the dilemma of identifying the taxonomic resolution necessary for a given study while remaining within the constraints of expertise, time, funding, and the analytical strategy.

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M.F. Bowman and R.C. Bailey.[1] Department of Zoology, University of Western Ontario, London, ON N6A 5B7, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: drbob@uwo.ca

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Table 1. Published studies used in analysis of the effect of taxonomic resolution on multivariate description of variation in benthic macroinvertebrate communities.

Author(s)	Community variation described	No. of communities (sites × times)	No. of taxa identified at genus or species level
Al-Safadi (1991)	Highlands versus lowlands of stagnant water wadis	4	35
Beiser et al. (1991)	Association with leaf packs	4	23
Crawford et al. (1992)	Implementation of advanced treatment of municipal wastewater	7 (three data sets)	47, 49, 50
Harris et al. (1992)	Impact of extensively treated wastewater from a pulp and paper mill (also had one qualitative data set)	10	25, 50
Short et al. (1991)	Gradient of chloride salts leeched from oilfields	7	45
Soster and McCall (1990)	Experimental field disturbances	32	13
Stewart and Haynes (1994)	Invasion of zebra mussels	8 (two data sets)	52, 44

There have been conflicting views on the level of taxonomic resolution necessary for community studies in freshwater and marine benthic research, possibly because of the greater diversity at higher taxonomic levels in marine systems. Some researchers have advocated species-level identification in the context of freshwater monitoring of water quality (Resh and Unzicker 1975). Others show that phylum-level identifications have been successful in detecting human impacts on marine benthic communities (Warwick 1988*a*, 1988*b*). Because there are so many reasons why a community will vary in composition, it is not surprising that conflicting, study-specific conclusions concerning necessary taxonomic resolution have arisen (Ferraro and Cole 1990).

In this study, we analyzed published data sets at varying levels of taxonomic resolution to illustrate how much (if any) information is lost by using higher level identification in studies of the spatial and temporal variation of freshwater benthic macroinvertebrate communities. Our goal was to quantify how much a description of community variation changes as taxonomic resolution changes.

Methods

A literature search for studies of freshwater benthic invertebrate communities included perusal of papers in various journals (*Canadian Journal of Fisheries and Aquatic Sciences*, *Ecology*, *Freshwater Biology*, *Hydrobiologia*, *Journal of Freshwater Ecology*, *Journal of the North American Benthological Society*, *Limnology and Oceanography*, and *New Zealand Journal of Marine and Freshwater Research*) published since 1990. Published data from 10 studies with identification to species or genus level, at least four communities (i.e., sites at the same time, times at the same site) sampled and at least 12 taxa were analyzed (Table 1).

NTSYS (Rohlf 1989) was used to analyze the data sets chosen from the literature search. For quantitative data sets (absolute or relative abundance of each taxon recorded), we used the reported taxon abundances as descriptors, and calculated Bray–Curtis and Euclidean distances (Rohlf 1989) between pairs of communities sampled within a given study, either at different places or different times at the same place. The resulting distance matrices are the "raw material" of multivariate analysis (ordination or clustering). It is the change in these distance matrices, as the taxonomic resolution is changed, that we were interested in examining. Therefore, the distance matrices were recalculated at decreasing levels of taxonomic resolution. Genera and species were successively grouped into families, orders, classes, and phyla with the aid of Pennak (1978), Merrit and Cummins (1984), and CSIRO (1991).

For one example study (Short et al. 1991), typical of the others, dendrograms from sequential agglomerative hierarchical nested (SAHN) cluster analyses (Rohlf 1989) and nonmetric multidimensional scaling (NMDS) ordination plots of the Bray–Curtis distance matrices illustrated differences in the descriptions of community variation at different levels of taxonomic resolution. This sample analysis illustrated graphically how changing taxonomic resolution affects multivariate analyses.

Mantel's matrix (Mantel 1967) comparison test (NTSYS; Rohlf 1989) was used to measure the correlation between distance matrices calculated from different levels of taxonomic resolution. This method measures the correlation between corresponding elements in two distance matrices. The test determines, for example, if the similarity between pairs of communities in a given study using generic taxonomy was correlated with the similarity between the communities using family-level taxonomy. Mantel's r correlation (Mantel 1967) coefficients were generated for all pairwise comparisons of taxonomic levels (e.g., genus–family, genus–class, etc.) for each published data set. The significance of a given correlation was determined by 10 000 random permutations of one of the matrices and generation of a null distribution of r values from these permutations. For a study of c communities (e.g., $c = 4$ in Beiser et al. 1991), there are $d = c(c-1)/2$ ($d = 6$ for Beiser et al. 1991) elements in the distance matrix and ${}_dP_d$ (= 720 for Beiser et al. 1991) possible permutations. Thus, if the observed matrix correlation is higher than any obtained from permutations, the significance of a particular Mantel's r (Mantel 1967) will only be as small as $1/{}_dP_d$ (= 0.0014 for Beiser et al. 1991).

To determine the effect of taxonomic resolution on qualitative (presence–absence) data, quantitative data were transformed to qualitative data at the genus level, and the aggregation of taxa and Mantel's test was carried out as previously described. For qualitative data, the Jaccard's distance measure (Rohlf 1989) was used.

Results

The community structure inferred by genus-level identifications in the published, quantitative data sets generally remained consistent when taxa were aggregated to higher levels. As an example typical of the consistency of community structure variation with varying taxonomic resolutions, in the Short et al. (1991) data set the 10 Mantel's correlations were between 0.886 and 1.000 (Table 2). Bray–Curtis distance matrices from this data set were used in SAHN cluster analysis, and the resulting dendrograms illustrated that the description of community structure remained consistent as taxa were aggregated (Fig.1). Two stream sites receiving oilfield brines, SC-1 and SF-2, were the first pair of communities clustered at all levels of taxonomic resolution, mainly because they were both dominated by the dipterans *Ephydra* (Ephydridae) and *Culicoides* (Ceratopogonidae). The low-diversity, transition community downstream of the brine-enriched sites, SF-3, was odd relative to the rest of the remaining sites at all taxonomic resolutions (Fig. 1). It had Culicoides in smaller numbers than SC-1

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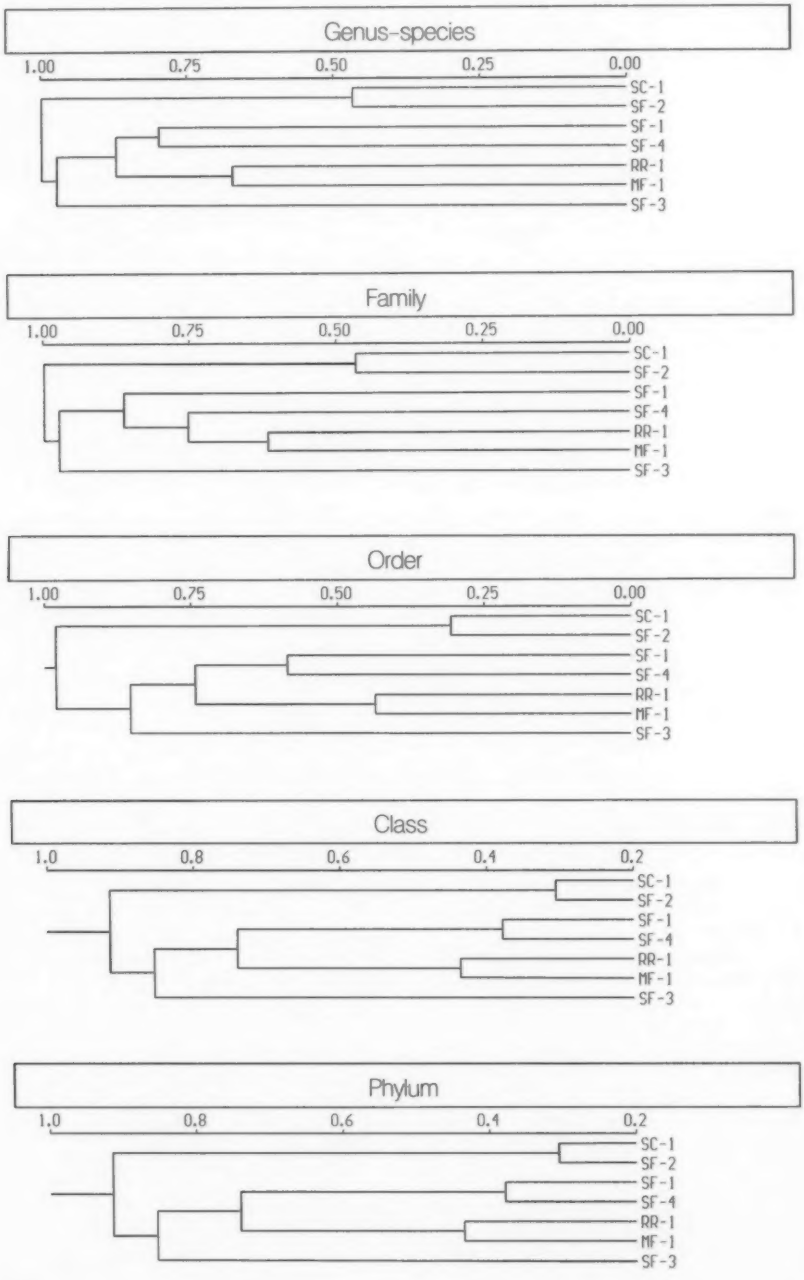
Fig. 1. SAHN cluster analysis on data from Short et al. (1991) based on a Bray–Curtis distance matrix calculated at five taxonomic resolutions (see Table 1).



and SF-2, but no *Ephydra*. The remaining four sites (SF-1, SF-4, RR-1, MF-1) all had more diverse communities that included various Diptera, Coleoptera, Megaloptera, Hemiptera, Ephemeroptera, Trichoptera, Plecoptera, and Odonata (Insecta), as well as Isopoda (Crustacea), Mesogastropoda (Gastropoda), Heterodonta (Bivalvia), and Haplotaxida (Oligochaeta). Similar, high correlations ($r >> 0.64$) between all levels of taxonomic resolution were observed for all but one (Table 5) of the quantitative data sets (Tables 3, 4, and 6–11).

There was a negative correlation ($r = -0.39$, $p << 0.0001$) between the number of genera or species reported in a given study and the average Mantel's r correlation among distance matrices from that study. More diverse communities showed less consistency in description of community variability as

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Table 2. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Short et al. (1991) quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.990***	0.953***	0.887***	0.887***
Family		0.956**	0.886***	0.886***
Order			0.941***	0.941***
Class				1.000***
Qualitative				
Genus–species	0.961***	0.790**	0.860**	$-0.087^{ns\ 0.47}$
Family		0.875***	0.816**	$0.057^{ns\ 0.38}$
Order			0.789*	$0.232^{ns\ 0.10}$
Class				$-0.080^{ns\ 0.49}$

Note: Correlations are labeled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; or ns, nonsignificant, e.g., $^{ns\ 0.47}$ ($p = 0.47$).

Table 3. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Al-Safadi (1991) quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.987*	0.929*	0.891*	0.891*
Family		$0.949^{ns\ 0.06}$	0.912*	0.912*
Order			$0.994^{ns\ 0.06}$	0.994*
Class				1.000*
Qualitative				
Genus–species	$0.302^{ns\ 0.41}$	$0.132^{ns\ 0.32}$	inv	inv
Family		$-0.492^{ns\ 0.32}$	inv	inv
Order			inv	inv
Class				inv

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns 0.41}$ ($p = 0.41$). inv, Data matrices were invariant at both scales of taxonomic resolution.

Table 4. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Beiser et al. (1991) quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	1.000*	0.924*	0.950*	0.951*
Family		0.924*	0.950*	0.951*
Order			0.879*	0.879*
Class				1.000*
Qualitative				
Genus–species	$0.796^{ns\ 0.09}$	$0.222^{ns\ 0.24}$	$0.571^{ns\ 0.11}$	inv
Family		$0.552^{ns\ 0.27}$	$0.093^{ns\ 0.45}$	inv
Order			$-0.639^{ns\ 0.08}$	inv
Class				inv

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; or ns, nonsignificant, e.g., $^{ns\ 0.45}$ ($p = 0.45$). inv, Data matrix was invariant at this scale of taxonomic resolution.

Table 5. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Crawford et al. (1992) No. 1 quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.772***	0.806***	$0.294^{ns\ 0.17}$	$0.273^{ns\ 0.17}$
Family		0.966***	$0.053^{ns\ 0.38}$	$0.039^{ns\ 0.40}$
Order			$0.097^{ns\ 0.32}$	$0.076^{ns\ 0.33}$
Class				0.998**
Qualitative				
Genus–species	0.353*	0.666**	0.761***	$0.376^{ns\ 0.06}$
Family		0.686**	$0.288^{ns\ 0.11}$	$0.108^{ns\ 0.24}$
Order			0.625**	0.410*
Class				0.500**

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.40}$ ($p = 0.40$).

Table 6. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Crawford et al. (1992) No. 2 quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.991**	0.970***	0.767**	0.807***
Family		0.993***	0.786***	0.815**
Order			0.786***	0.807***
Class				0.982***
Qualitative				
Genus–species	0.822**	0.713**	$0.685^{ns\ 0.06}$	$0.359^{ns\ 0.32}$
Family		0.842*	0.873*	$0.536^{ns\ 0.28}$
Order			0.913*	$0.603^{ns\ 0.15}$
Class				$0.728^{ns\ 0.14}$

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.32}$ ($p = 0.32$).

Table 7. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Crawford et al. (1992) No. 3 quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.950***	0.944***	0.734**	0.735**
Family		0.990**	0.674***	0.676**
Order			0.643**	0.646**
Class				1.000***
Qualitative				
Genus–species	0.757**	0.682**	$0.030^{ns\ 0.38}$	$0.190^{ns\ 0.22}$
Family		0.860***	$0.130^{ns\ 0.31}$	0.389*
Order			$0.324^{ns\ 0.14}$	$0.373^{ns\ 0.12}$
Class				$-0.161^{ns\ 0.35}$

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.38}$ ($p = 0.38$).

taxa were pooled into higher categories. There was no correlation ($r = 0.13$, $p = 0.21$) between the number of communities sampled in a study and the average Mantel's r correlation between pairs of distance matrices from that study.

For data transformed from a quantitative to a qualitative scale, we saw less consistency across levels of taxonomic resolution in the description of community structure (Tables 3–1), particularly for nonadjacent comparisons (e.g., genus–order). There were even cases of negative correlations between distance matrices calculated at different levels of taxonomic resolution.

At the class and phylum levels, several data sets had "invariant" distance matrices (i.e., all classes or phyla were

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Table 8. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Harris et al. (1992) quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.941***	0.847***	0.809***	0.694***
Family		0.893***	0.900***	0.679***
Order			0.894***	0.700**
Class				0.746***
Qualitative				
Genus–species	0.742***	0.298*	$0.184^{ns\ 0.09}$	$0.184^{ns\ 0.10}$
Family		0.525***	0.255*	0.255*
Order			0.332*	0.332*
Class				1.000**

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.10}$ ($p = 0.10$).

Table 9. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Soster and McCall (1990) quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	0.977***	0.934***	0.934***	0.930***
Family		0.966***	0.966***	0.959***
Order			1.000***	0.994***
Class				0.994***
Qualitative				
Genus–species	$0.757^{ns0.10}$	$0.672^{ns\ 0.10}$	$0.672^{ns\ 0.10}$	$0.634^{ns\ 0.10}$
Family		$0.854^{ns\ 0.10}$	$0.854^{ns\ 0.10}$	$0.832^{ns\ 0.10}$
Order			$1.000^{ns\ 0.10}$	$0.994^{ns\ 0.10}$
Class				$0.994^{ns\ 0.10}$

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.10}$ ($p = 0.10$).

Table 10. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Stewart and Haynes (1994) No. 1 quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	1.000***	0.998***	0.998***	0.997***
Family		0.999***	0.998***	0.997***
Order			0.999***	0.998***
Class				0.999***
Qualitative				
Genus–species	0.647***	0.470**	0.644**	0.492*
Family		0.892**	0.676**	0.636*
Order			0.687***	0.668*
Class				0.713*

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; or *, $p < 0.05$.

Table 11. Correlations between distance matrices calculated at varying levels of taxonomic resolution from the Stewart and Haynes (1994) No. 2 quantitative and qualitative data sets.

	Family	Order	Class	Phylum
Quantitative				
Genus–species	1.000***	1.000***	0.998***	0.996**
Family		1.000***	0.999***	0.997***
Order			0.999***	0.998***
Class				0.999***
Qualitative				
Genus–species	0.621***	$0.343^{ns\ 0.08}$	0.666**	0.782**
Family		0.543**	0.662**	0.657*
Order			0.674**	0.397*
Class				0.688**

Note: Correlations are labelled as significant at ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$;or ns, nonsignificant, e.g., $^{ns\ 0.08}$ ($p = 0.08$).

present in all communities, so all communities were identical at this resolution). This made it impossible to correlate such distance matrices with others based on order, family, or genus taxonomy. This phenomenon does, however, highlight an obvious limitation of qualitative data in detecting community variation.

In contrast to the quantitative data sets, for qualitative data there was no tendency for higher correlations among distance matrices to occur in taxa-rich studies ($r = -0.056$; $p = 0.35$). Presence–absence studies incorporating more communities, however, tended to have more consistent (i.e., higher Mantel's r (Mantel 1967) correlation between distance matrices) variation among communities across levels of taxonomic resolution ($r = 0.36$; $p << 0.0001$).

Discussion

The consistency of the description of community variation across different levels of taxonomic resolution reinforces the conclusions of Warwick (1988*a*, 1988*b*) in his determination of the taxonomic discrimination required to detect pollution effects in marine communities. In both studies, Warwick concluded that data aggregated to family level were no less informative than species-level data. Also, phylum-level community descriptions were at least as correlated with the actual pollution gradient as those based on species identifications and more often had better correspondence.

It has been suggested that community responses to human perturbations may be more easily detected against a background of natural variability in factors such as water depth and sediment size by looking at higher taxonomic levels (Warwick 1993). This implies that some of the variation seen in the abundance of individual species resulting from adaptations to a narrow range of natural environmental conditions will not be reflected at higher taxonomic levels, and the effects of pollution will be more evident. Similarly, data "noise" at the species level due to biogeographic variability may be reduced with analyses at higher taxonomic levels.

Community change through time resulting from increased stress (due to human or other forces) may be manifested at higher and higher levels of biological organization, such that the taxonomic level necessary and sufficient to assess change increases in a step function (Ferraro and Cole 1990). For example, one would need to identify organisms to species to detect a subtle environmental change that only affected one species. But a more general stress that eliminated all the genera in a given family could be detected by identifying organisms in the community to family level only (Ferraro and Cole 1992). Pearson and Rosenberg (1978) first proposed this idea of hierarchic response to stress and described the step function

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that may explain the taxonomic resolution required to detect a human impact on a benthic community. Crawford et al.'s (1992) description of the recovery of benthic invertebrate communities following implementation of advanced treatment of municipal wastewater appeared to follow this pattern. We found that the community structure described by identification of genera was only maintained up to ordinal-level for the control site, whereas the two impacted sites could be described equally well with genus-level or phylum-level identification.

The value of qualitative, relative to quantitative, data is unclear. Our results suggested that as taxa are aggregated, qualitative data fail to consistently retain the original description of variation in community structure derived from genus-level identifications. Marchant (1990) found that, when qualitative data were aggregated to the family level or fewer than four replicates were taken at a given site, distortions in ordinations occurred. Our results suggest that, if trade-offs are necessary for a given study of community variation as they usually are, it is better to sacrifice taxonomic resolution than quantitative data in gathering such data.

There are, of course, situations in both pure and applied studies where coarse taxonomic resolution is not an option. Evaluation of known indicator species can reduce the cost of impact assessment. In marine research, the "quality" of the environment has been assessed by the presence or absence of certain indicator species (Resh and Unzicker 1975; Pocklington and Wells 1992).

Careful consideration of necessary and appropriate taxonomic resolution has the potential to improve the multivariate description of benthic community variation in space and through time and, particularly, in response to pollution. It has been suggested that species identification of freshwater macroinvertebrates is essential for applied studies, since there can be considerable variation from species to species within a taxon with respect to their pollution tolerances (Resh and Unzicker 1975). Our findings illustrate that species identification of known indicator species could effectively be coupled with multivariate community analysis using relatively coarse taxonomic resolution (e.g., family or order). Through phylum-level meta-analysis of marine systems, Warwick and Clarke (1993) have developed a multivariate method for analyzing pollution effects that seems to have global generality in marine systems. Analogous studies in freshwater systems are needed to assess the appropriate taxonomic resolution for detecting various amounts and kinds of pollution in different habitats.

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Differences in sexual maturity and fall emigration between diploid and triploid brook trout (*Salvelinus fontinalis*) in an Adirondack lake

Jennifer A. Warrillow, Daniel C. Josephson, William D. Youngs, and Charles C. Krueger

Abstract: High levels of emigration coincident with maturity and spawning have been reported from brook trout (*Salvelinus fontinalis*) populations in Adirondack lakes. These lakes typically had few spawning areas and required stocking to maintain populations. We compared diploid and triploid brook trout to identify differences in gonadal development and emigration. Age 1+ and 2+ diploid and triploid brook trout held in captivity were examined internally for gonadal development. More diploid trout were mature than triploid fish ($p < 0.01$). Of triploid brook trout that matured, all were males. Yearling diploid and triploid brook trout were also stocked into a lake that had an outlet but no spawning areas. During the fall spawning season, only mature yearling triploid males, diploid males, and diploid females were caught in an outlet trap. No triploid females were caught. A greater proportion of diploids emigrated than triploids ($p < 0.01$). Triploidy in females arrested emigration by preventing sexual maturation. Triploid male brook trout should not be stocked because they can pose a reproductive risk to wild brook trout downstream from lakes. Stocking triploid females could reduce fall emigration and thus reduce the loss of catchable brook trout from Adirondack lakes with outlets and little spawning habitat.

Résumé : Des degrés élevés d'émigration coïncidant avec la maturité et le frai ont été signalés dans le cas de population d'ombles de fontaine (*Salvelinus fontinalis*) vivant dans les lacs des Adirondacks. De manière typique, ces lacs étaient pauvres en aires de frai et devaient être ensemencés pour assurer le maintien des populations. Nous avons comparé des ombles de fontaine diploïdes et triploïdes pour étudier les différences au niveau du développement des gonades et de l'émigration. Des ombles diploïdes et triploïdes d'âge 1+ et 2+ gardés en captivité ont fait l'objet d'un examen interne pour vérifier le développement des gonades. Les individus matures étaient plus nombreux chez les diploïdes que chez les triploïdes ($p < 0{,}01$). Les individus triploïdes qui sont parvenus à maturité étaient tous des mâles. Des ombles de fontaine diploïdes et triploïdes de l'année ont été ensemencés dans un lac qui possédait un émissaire, mais qui était dépourvu d'aires de frai. Au cours de la saison de frai, seuls des mâles triploïdes de l'année mature, des mâles diploïdes, des femelles diploïdes ont été capturés dans l'émissaire. Aucune femelle triploïde n'a été capturée. Chez les émigrants, on a constaté une plus forte proportion de diploïdes que de triploïdes ($p < 0{,}01$). La triploïdie chez les femelles a enrayé l'émigration en empêchant la maturation sexuelle. On ne devrait pas ensemencer d'ombles de fontaine mâles triploïdes parce qu'ils peuvent constituer un risque reproductif pour les ombles de fontaine sauvages vivant en aval des lacs. L'ensemencement de femelles triploïdes pourrait réduire l'émigration d'automne et, ainsi, réduire la perte d'ombles exploitables dans les lacs des Adirondacks possédant des émissaires, mais peu d'habitats de frai.
[Traduit par la Rédaction]

Introduction

Large-scale fall emigration of mature brook trout (*Salvelinus fontinalis*), coincident with spawning season, was reported from stocked lakes with outlets in the Adirondack region of New York State (Josephson and Youngs 1996). The stocked lakes had few spawning areas, and brook trout probably had emigrated in search of suitable spawning sites. Fall emigration was associated with sexual maturity and represented 37–69% potential losses from the stocked populations. Thus, the trout fisheries in these lakes could have been severely affected. If maturity could be suppressed or eliminated in the brook trout used for stocking, losses from fall emigration likely would be reduced and fisheries improved. Extensive evidence suggests that triploidy reduces or prevents maturation in brook trout (Allen and Stanley 1978) and other salmonids (Thorgaard and Gall 1979; Lincoln and Scott 1984; Benfey et al. 1989; Benfey 1991). Therefore, triploid brook trout rather than diploids could be stocked to reduce fall emigration losses and enhance put–grow–take fisheries in similar lakes.

The purpose of this study was to compare the fall emigration of diploid and triploid brook trout stocked into a small lake that had an outlet but no spawning areas. The objectives were to determine (*i*) if triploidy reduced gonadal development

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J.A. Warrillow,[1] D.C. Josephson, W.D. Youngs, and C.C. Krueger.[2] Adirondack Fishery Research Program, Department of Natural Resources, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, U.S.A.

[1] Present address: 801 Claytor Square, Blacksburg, VA 24060, U.S.A.
[2] Author to whom all correspondence should be addressed.

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Table 1. Sex and maturity of diploid and triploid yearling brook trout stocked fall of 1993 into Mountain Pond, New York.

Ploidy	Number stocked	Unknown (immature)	Female		Male	
			Ripe	Green	Ripe	Green
2*n*	110	35	0	26	45	4
3*n*	95	58	0	0	0	37

Note: Maturity was assessed by external examination.

or otherwise inhibited sexual maturation and (*ii*) whether triploid trout emigrated less than diploid trout.

Materials and methods

Strains, ploidy manipulation, and stocking

Triploid and diploid brook trout were produced at the Little Moose Field Station of the Adirondack Fishery Research Program, located near Old Forge, New York. Temiscamie (female) × domestic (male) interstrain crosses were used in this study. The domestic strain matures at ages 0 or 1. This strain originated from several thousand Rome strain fingerlings that were transferred from the state fish hatchery in Rome, N.Y., in 1979 and held at the Little Moose Field Station for several generations (see Rome strain description in Perkins et al. 1993). The Temiscamie strain matures at ages 1–3 and originated from gametes collected from 20–30 adults captured in the fall each year in 1965 and 1967 from the Temiscamie River, Quebec. Adults captured were presumed to be upstream migrants from a population in Lake Albanel 128 km downstream. This strain was subsequently held and propagated in Adirondack ponds in New York (Van Offelen et al. 1993).

Triploid trout were created in early November 1991 by heat shocking diploid zygotes 10 min after fertilization for 10 min at 28°C (Scheerer and Thorgaard 1983). Each ploidy lot was created from the same collection of gametes from 30 adults (1:1 sex ratio); five males were used to fertilize the eggs from each female. Trout were marked with jaw tags and blood samples were drawn from each fish in June 1993. The blood samples were tested by flow cytometric analysis of erythrocytes to determine the ploidy of each individual (Allen 1983). Blood drawn from the caudal peduncle was dispensed (1 µL) into 300 µL of a stain solution of 0.05 mg/mL propidium iodide and 0.1% sodium citrate. Analysis of the samples was performed on an Epics Profile flow cytometer (Epics Division of Coulter Corporation, Hialeah, Fla.). Diploid brook trout blood was used as a comparative standard to presumed triploids. Based on the results of this analysis, diploid trout were discarded to create a 100% triploid lot. Diploid and triploid trout were reared under identical conditions until stocking in October 1993.

One-hundred-ten yearling diploid and 95 yearling triploid brook trout, from the 1992 year-class, were stocked into Mountain Pond on 1 October 1993 (mean diploid length 194 mm, range 145–235 mm; mean triploid length 184 mm, range 155–226 mm). Mountain Pond (43°42′N, 75°52′W) is a 3.4-ha lake with an outlet, located in the southwest region of the Adirondack Park, New York State. Three days prior to stocking, maturity of the fish was assessed by external examination. In addition, approximately 60 diploid and triploid trout were not stocked but retained in the hatchery for later internal assessment of maturity and gonadal development.

Assessment of maturity and gonadal development

External assessment of maturity was based on body shape, coloration, and gamete ripeness. Each fish stocked and those that emigrated were classified as immature, green, ripe, or spent. Fish that did not appear ready to spawn and showed no bright coloration were classified as immature. Green fish were those that had body cavities rounded with gametes (females) or were brightly colored (males) but that did not extrude gametes. Fish that extruded gametes when gently squeezed were categorized as ripe. Spent fish had flaccid body cavities that appeared as if they had been distended with gametes but were now empty.

Internal assessment of gonadal development was performed over two spawning seasons (1993 and 1994) on fish not stocked but held in the hatchery. Twenty-one diploids and 20 triploids from the 1992 year-class (age 1+) were frozen on 4 October 1993 and dissected 3 days later. Twenty-six diploid and 28 triploid age 2+ fish from the 1992 year-class were dissected on 7 November 1994. Based on dissection, gonads of fish were classified as either undeveloped, fully developed, or partially developed. Undeveloped gonads were small, showed no maturation or development, appeared stringy, and sex was indeterminable. Fully developed gonads were developed to the point of ripeness or near ripeness, and sex was easily recognized. Eggs in these fish were large and testes were white and long. Sex of gonads classified as partially developed was recognizable, but eggs were small and testes were gray and shorter than fully developed gonads. Gonadosomatic indices (Vladykov 1956) were calculated from the dissected fish to compare gonadal development among diploids and triploids. The gonadosomatic index (GSI) was calculated as

$$\mathrm{GSI} = \frac{\text{gonad weight (both halves) (g)} \times 100}{\text{body weight (g)}}$$

Outlet trapping

Emigration of the stocked brook trout from Mountain Pond was monitored with an inclined screen fish trap (Wolf 1951) constructed across the outlet, located 100 m downstream from the lake. After stocking, the trap was tended once or twice weekly for a 2.5-month period, from 1 October to 15 December, 1993. Tag numbers were recorded from fish caught to determine their ploidy, and each fish was examined externally to assess maturity. Fish caught in the trap were given a lower caudal fin clip and released back into the lake. No additional data were recorded from trout recaptured at later dates.

Statistical procedures

Binomial proportion tests (π; decision level = 0.01) were used to compare the ploidy and maturity proportions of fish kept in the hatchery and of those that were stocked and subsequently emigrated. The null hypotheses for these tests were the proportion of diploid fish that matured was equal to the proportion of triploid fish that matured (just prior to stocking), the proportion of diploid fish that emigrated was equal to the proportion of triploid fish that emigrated, and the proportion of mature diploid fish that emigrated was equal to the proportion of mature triploid fish that emigrated. These tests presumed that the sex ratio between ploidy lots was similar and that mortality was similar between diploid and triploid trout for the 2.5-month period after stocking. Chi-square tests of independence (χ^2; decision level = 0.01) were used to determine whether maturity and emigration, and ploidy and emigration, were independent. Two sample *t*-tests (unequal variance) were conducted to compare GSI values between ploidies of the same gender and age.

Results

Diploid versus triploid maturity

Maturity of the brook trout to be stocked, externally assessed 3 days prior to release, revealed that the proportion of the diploid lot already mature (0.68; green or ripe) was higher than that of the triploid lot (0.39) (π, $p < 0.001$; Table 1). All triploid males were classified as green, and most displayed external coloration typical of ripe diploid males. No triploid females, either green or ripe, were identified by external examination; however, green diploid females were observed. A larger number of triploids ($n = 58$) than diploids ($n = 35$)

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Table 2. Gonadal development and mean GSI of diploid and triploid age 1 and 2 brook trout reared in the hatchery and dissected in October 1993 and in November 1994.

Ploidy	Sex	Age	Gonadal development	Mean GSI	Sample size
			October 1993		
Diploid	Unknown (immature)	1	Undeveloped	na	1
Diploid	Male	1	Fully developed	2.96	9
Diploid	Female	1	Fully developed	10.6	5
Diploid	Female	1	Partially developed	0.53	6
Triploid	Unknown (immature)	1	Undeveloped	na	11
Triploid	Male	1	Partially developed	2.04	9
			November 1994		
Diploid	Female	2	Fully developed	14.0	9
Diploid	Male	2	Fully developed	2.58	17
Triploid	Female	2	Partially developed	0.09	10
Triploid	Male	2	Partially developed	0.53	18

Note: Maturity was assessed by internal examination. GSI was not available (na) for immature fish (sex undetermined) because gonadal tissue was not distinct.

Table 3. Maturity level of female and male yearling brook trout that had emigrated from Mountain Pond and were caught in the outlet trap during the fall 1993.

		Female				Male			
	Immature	Green	Ripe	Spent	Total	Green	Ripe	Spent	Total
N_{EM}[a]	0	0	9	3	12	9	25	6	40
Percent[b]	0	0	17	6	23	17	48	12	77

[a] N_{EM}, number of emigrants caught.
[b] Percentage of total emigrants.

were identified as immature (i.e., sex could not be determined).

Differences in maturity, related to sex and ploidy, were observed based on internal examination of brook trout from the two lots retained in the hatchery and not stocked (Table 2). Among age 1+ fish examined in 1993, only 1 diploid fish of 21 had undeveloped gonads in comparison with 11 of 20 triploid fish examined. All yearling male and female fish ($n = 14$) with fully developed gonads were diploids. Nine triploid males and six diploid females had partially developed gonads. All triploids were classified as either male or immature; no triploid females were identified. All diploid males were ripe, and all triploid males were green. The next year (1994) as age 2+ fish, all 26 diploid fish had fully developed gonads and the gonads of all 28 triploid fish were partially developed. All age 2+ triploid females had partially developed but small ovaries.

Diploid GSI measurements were always greater than triploid GSI measurements in same-sex comparisons of age 1 and 2 trout held in the hatchery (Table 2). Yearling diploid male GSI values were different from triploid male GSI values (t-test, $p \cong 0.00$). Similar differences were observed the next year between GSI values for diploid and triploid age 2 males (t-test, $p < 0.001$) and between diploid and triploid age 2 females (t-test, $p < 0.001$).

Emigration

Fall emigration and maturity of brook trout were dependent (χ^2, $p < 0.01$; Table 3). Only mature brook trout were caught in the outlet trap in the fall of 1993. Most fish (77%) that emigrated were males. Ploidy and emigration of trout were also dependent (χ^2, $p < 0.01$; Table 4). Based on the total number of each lot stocked, a greater percentage of diploid trout (32%) emigrated from Mountain Pond than triploids (17%; Table 4; π, $p < 0.001$). However, when only the number of mature fish stocked within each lot was considered, the percentage of diploid (47%) and triploid (43%) trout that emigrated was not statistically different (π, $p > 0.05$; Table 4).

Table 4. Ploidy of brook trout that emigrated as yearlings from Mountain Pond during the fall 1993 and caught in an outlet trap.

			Emigrants			
Ploidy	No. stocked (N_s)	No. mature at stocking (N_m)	No. (N_{EM})	Percent male	Percent of total stocked[a]	Percent of total mature at stocking[b]
2*n*	110	75 (68%)	36	67	33	48
3*n*	95	37 (39%)	16	100	17	43

[a] Percent of total stocked = N_{EM}/N_s.
[b] Percent of total mature at stocking = N_{EM}/N_m.

Discussion

Comparisons of diploid and triploid brook trout of the same sex and age demonstrated that differences in maturity and gonadal development were due to ploidy. Gonads in age 1 and 2 diploid brook trout were significantly larger than those in triploid fish. After this study was concluded, triploid brook trout from the next year-class (1993) were held until age 3+, and no mature triploid females were observed, which is consistent with observations by others (e.g., Boulanger 1991). Triploidy seems to prevent maturation in female brook trout at least through age 3.

Triploid brook trout were less likely to emigrate in the fall than diploid trout. However, when only mature fish were

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considered, ploidy had little effect on emigration tendency. Mature triploids (and only males matured) emigrated at approximately the same proportion as mature diploids (both males and females matured) after being stocked into Mountain Pond. Because no mature triploid females were observed, most of the immature triploids were probably females. The absence of emigration by immature triploids (probably females) supported the idea that suppression of maturity through triploidy would reduce fall emigration by brook trout.

Triploid male brook trout displayed the same secondary sexual traits and had similar but smaller testes at ages 1 and 2 as diploid males. Likewise, Thorgaard and Gall (1979) reported that triploid male rainbow trout (*Oncorhynchus mykiss*) had partially developed testes similar to diploid males in appearance. Testes of triploid rainbow trout were grayer, firmer, and slightly smaller than testes of diploids, and triploids had a lower GSI (Lincoln and Scott 1984). Benfey et al. (1989) reported similar differences in gonadal development for male diploid and triploid rainbow trout and pink salmon (*Oncorhynchus gorbuscha*). Absence of ripe triploid males in our study was probably due to an absence of sperm ducts in these fish, and thus, gametes could not be extruded (Boulanger 1991). In rainbow trout, the presence of a third set of chromosomes resulted in abnormal (aneuploid) sperm, if sperm was formed at all (Benfey et al. 1986). Despite the differences in gonadal development between diploid and triploid males, males of both ploidies exhibited similar emigration from the lake.

No female triploid brook trout were identified in this study by external characteristics at spawning time; however, females which had small, undeveloped, stringy ovaries were identified by dissection. Similar undeveloped ovaries were observed in triploid female rainbow trout (Lincoln and Scott 1984; Thorgaard and Gall 1979). In a comparative study of female diploid and triploid rainbow trout and pink salmon, the triploids did not mature, and a lack of oogenesis was common, perhaps as a result of abnormal endocrine function (Benfey et al. 1989). These studies explain the absence of externally identifiable triploid females observed in our study. Further, the differences in gonadal development we observed between diploid and triploid females were also associated with behavioral differences. Emigration from the lake was exhibited by yearling diploid females but not by yearling triploid immature fish presumed to be mostly females. In 1994 when the fish in this study were age 2+, no water flowed in the outlet of Mountain Pond until late in the spawning season (3 November) owing to a drought. However, six fish of the 1992 year-class were caught in the outlet trap, and all were diploids. Emigration of age 2+ triploid females also seems unlikely based on the lack of fully developed gonads observed in the age 2+ triploid fish held in the hatchery (Table 4).

Based on the results of our study, stocking triploid female brook trout could reduce fall emigration of age 1 and 2 fish from lakes with outlets. Many lakes in the Adirondack region have little or no natural reproduction of brook trout, and thus, their fisheries are dependent on stocking. Stocking of mixed-sex triploid lots of brook trout, male and female, as an approach to reduce fall emigration, however, is undesirable in two aspects. First, some male triploid brook trout did mature and emigrate from Mountain Pond, which compromised the effectiveness of the approach. Second, mature triploid male brook trout will likely exhibit spawning behavior and, therefore, could compete for females and disrupt normal spawning of wild fish (Boulanger 1991). Because triploid males will emigrate, stocking of these fish could be detrimental to wild populations that may exist downstream from lake outlets. To minimize these problems, only female triploid brook trout should be used for stocking when using ploidy as an approach to control emigration. Ultimately, this approach may be viable if all female triploid lines can be produced. All-female lots of brook trout can be produced by various sex reversal strategies (e.g., Galbreath et al. 1994).

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Greenland halibut (*Reinhardtius hippoglossoides*) in the North Atlantic are genetically homogeneous

Morgan L. Vis, Steven M. Carr, W.R. Bowering, and William S. Davidson

Abstract: Greenland halibut (*Reinhardtius hippoglossoides*) have become of great importance to the Canadian fishery since the moratorium on northern cod (*Gadus morhua*), and an understanding of stock relationships among populations at the northern and southern extremes of their commercial range is crucial for proper management. We compared mitochondrial DNA sequence variation among fish taken from thoughout the Northwest Atlantic (Flemish Pass, the Grand Banks, Davis Strait, and Northwest Greenland) with samples from the Gulf of St. Lawrence, Iceland, and Norway. Within a 401 base pair portion of the cytochrome *b* gene, 22 genotypes were identified. Three of these occur at frequencies >10% and in the same relative abundances in all samples (except the Gulf of St.Lawrence). Genotype proportions do not differ significantly among samples, and genetic subdivision among samples (measured by the coancestry coefficient θ) is nil. Genetic distances among samples are not related to geographic distribution: pairwise differences between the Gulf sample and other western Atlantic samples exceed those for trans-Atlantic comparisons. These data suggest that there is sufficient mixing of Greenland halibut, not only within the NAFO regulatory area, but among sites in the North Atlantic generally, to prevent the development or maintenance of genetically independent stocks.

Résumé : Le flétan du Groenland (*Reinhardtius hippoglossoides*) est devenu une espèce très importante pour la pêche canadienne depuis que le moratoire a été décrété sur la pêche à la morue (*Gadus morhua*) et, pour assurer la bonne gestion de cette espèce, il est indispensable de connaître les relations entre les populations occupant les extrémités nord et sud de l'aire de répartition commerciale de l'espèce. Nous avons comparé la variation de la séquence d'ADN mitochondrial chez des poissons capturés dans l'Atlantique Nord-Ouest (col Flemish, talus continental au nord-est de Terre-Neuve, détroit de Davis et au nord-ouest du Groenland) avec des échantillons provenant du golfe du Saint-Laurent, d'Islande et de Norvège. On a identifié 22 génotypes dans un segment de 401 paires de bases du gène du cytochrome *b*. Trois de ces génotypes ont une fréquence >10% et présentent les mêmes abondances relatives dans tous les échantillons (sauf pour les poissons du golfe du Saint-Laurent). Les proportions génotypiques n'ont pas différé de manière statistiquement significative entre les échantillons. La subdivision génétique entre les échantillons était nulle et les distances génétiques parmi les échantillons n'étaient pas liées à la répartition géographique : les différences touchant les paires entre les échantillons du golfe et d'autres échantillons de l'Atlantiqe Ouest étaient supérieures à celles que l'on a observées dans les comparaisons transatlantiques. Ces données indiqueraient qu'il y a suffisamment de mélange chez le flétant du Groenland, non seulement pour ce qui est de l'ensemble de l'Atlantique Nord-Ouest, mais dans les différents sites situés dans l'Atlantique Nord de façon générale, pour empêcher la constitution ou le maintien de stocks génétiquement indépendants.

Introduction

Greenland halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic are widely distributed from high in the Arctic between Canada and Greenland to as far south as the Scotian Shelf; they are most abundant from the eastern Grand Bank and Flemish Cap well beyond the Canadian 200-mile (1 mile = 1.609 km) limit and north (Bowering and Brodie 1995) (Fig. 1). It is a deepwater species with higher densities occurring in depths of about 500–1200 m along the edge of the continental slope and the channels running between the fishing banks of the continental shelf as well as the deep inshore bays of eastern Newfoundland and the fjords of Greenland and Baffin Island. In recent years, with advances in modern fishing technology, it has been found to be commercially abundant in some areas as deep as 1500–1800 m, particularly in the proximity of the Sackville Spur and Flemish Pass, immediately east of the Grand Banks (Bowering and Brodie 1995) and has been caught in longline investigations as deep as 2200 m off West Greenland (Boje and Hareide 1993) and the Flemish Cap (de Cardenas et al. 1996). The species is also of commercial importance in the Gulf of St. Lawrence and exists in limited quantities along the south Newfoundland coast, Fortune Bay, and in the Laurentian Channel.

Stock structure of Greenland halibut in the Northwest Atlantic has been studied using a variety of methods (e.g., protein electrophoresis (Fairbairn 1981), parasites (Khan et al. 1982; Arthur and Albert 1993), morphometrics (Bowering 1988),

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M.L. Vis[1] **and S.M. Carr.**[2] Genetics, Evolution, and Molecular Systematics Laboratory, Department of Biology, Memorial University of Newfoundland, St. John's, NF A1B 3X9, Canada.
W.R. Bowering. Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.
W.S. Davidson. Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF A1B 3X9, Canada.

[1] Present address: Department of Botany, Ohio University, Athens, Ohio 45701, U.S.A.
[2] Author to whom all correspondence should be addressed. e-mail: scarr@morgan.ucs.mun.ca

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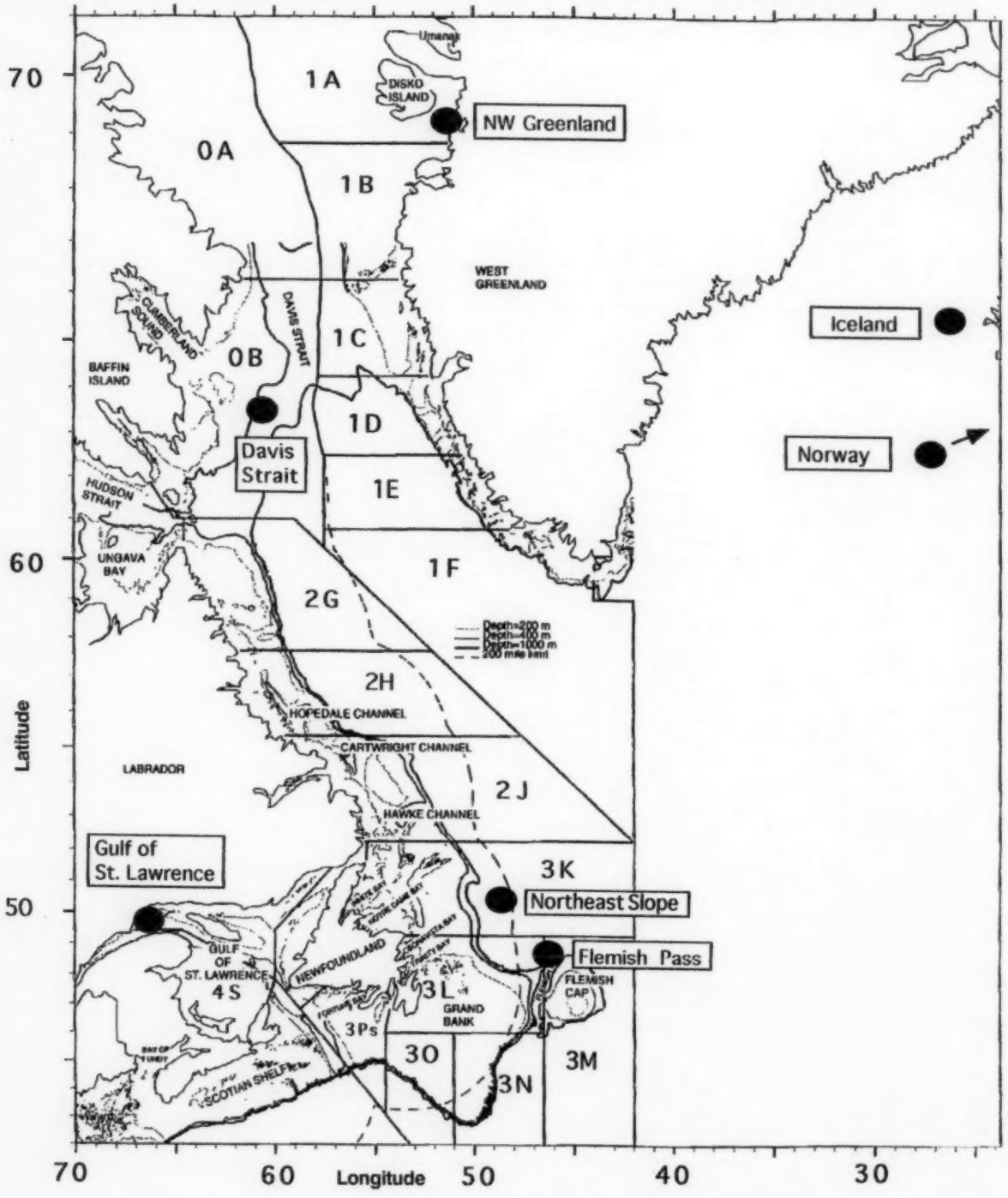
Fig. 1. Sampling locations from which Greenland halibut were collected. Northwest Atlantic Fisheries Organization (NAFO) divisions, depth contours, and the 200-mile Canadian economic limit are shown for reference. The base map is taken from Bowering and Brodie (1995).



meristics (Misra and Bowering 1984; Riget et al. 1992), and tagging (Bowering 1984; Riget and Boje 1989)). These data suggest that Greenland halibut comprise a single self-sustaining stock from Davis Strait to the Grand Bank and Flemish Cap (Bowering and Chumakov 1989). Greenland halibut in the Gulf of St. Lawrence are believed to be a separate, relatively

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small, self-sustaining stock, which receives migrants from the Labrador area through the Strait of Belle Isle between Quebec and the island of Newfoundland (Bowering 1982). The major spawning location of Greenland halibut in the western Atlantic is in the deep slope area of Davis Strait along the boundary between Canada and Greenland (Smidt 1969; Templeman 1973; Chumakov 1975; Bowering 1983). However, with research activity on Greenland halibut expanding to very deep water, complemented by commercial fishing operations, additional information on spawning locations is becoming available. Fish in spawning condition have recently been reported from the deep slope area off the coasts of Labrador and northeastern Newfoundland by domestic gillnetters (Morgan and Bowering 1996) to as far south as Flemish Pass (Junquera and Zamarro 1994; Morgan and Bowering 1996). It is not clear whether this is a change in behaviour or a typical behaviour that has not been previously observed, although major shifts in Greenland halibut distribution have been recorded in recent years (Anonymous 1995). Apparent changes in behaviour have been reported also for other flatfish species in this area such as witch flounder (Bowering 1995) and American plaice (*Hippoglossoides platessoides*) (Bowering et al. 1995*a*, 1996, 1997; Brodie et al. 1995). The extent of Greenland halibut spawning in the area of the continental slope off eastern Canada has been discussed in Morgan and Bowering (1997).

As a result of the massive increase in fishing effort by foreign vessels directed towards Greenland halibut in the Northwest Atlantic Fisheries Organization (NAFO) Regulatory Area during the 1990s, annual catches in this area alone have nearly doubled the highest catch ever recorded in traditional fisheries since the Greenland halibut fishery began more than 30 years ago (Bowering and Brodie 1995). Annual catches from West Greenland to the Flemish Pass averaged about 85 000 t during 1990–1994, making it the largest groundfish fishery in the Northwest Atlantic (Anonymous 1995). As a consequence of the collapse of the West Greenland cod fishery and most other Canadian Atlantic groundfish fisheries, the offshore fishery for Greenland halibut in the Davis Strait in the 1990s has also increased substantially (Jørgensen and Boje 1995).

Several disturbing trends have accompanied these new fisheries. There have been steep systematic declines in estimates of stock size from Davis Strait to the Grand Bank and Flemish Pass (Bowering and Brodie 1995). Canadian catches of Greenland halibut have declined dramatically in traditional fishing areas and effort has been moving progressively deeper throughout the distribution range. Finally, Greenland halibut of commercial size have virtually disappeared in traditional midshore areas despite no apparent decline in recruitment (Bowering et al. 1995*b*).

In consequence of its change in status from an "underutilized species" to the major groundfish fishery in the western Atlantic, Greenland halibut have received a very high public profile. A serious international dispute regarding the fishery arose between Canada and the European Union during 1995, which received much attention at the United Nations Conference on High Seas Fishing. This level of interest will likely continue until a more conclusive evaluation of stock structure is available. In common with other fisheries, Greenland halibut in the Northwest Atlantic are regulated according to a system of statistical subareas and divisions established by NAFO (cf. Fig. 1). Fish stocks that fall within the 200-mile economic zone are typically managed unilaterally by Canada, whereas stocks that occur outside or overlap that limit fall under the multinational control of the NAFO Fisheries Commission. Canadian managers are particularly concerned about the relationship of Greenland halibut outside the 200-mile economic limit on the Grand Banks of Newfoundland and adjacent waters (NAFO Subarea 3) to those within the Canadian economic zone as far north as Davis Strait. They ask whether Greenland halibut outside the 200-mile limit on the Grand Banks and adjacent waters constitute a separate stock, or do they instead comprise an extension of the distribution from Davis Strait southward along the deep edge of the continental slope, as currently believed? Are the newly found spawning fish in the deepslope areas of the NAFO Regulatory Area migrants from the Canadian zone? The implications for fisheries management can be quite complex considering the possible scenarios for stock structure. Prior to 1995, Greenland halibut in the far north in the Davis Strait and northwestern Greenland (NAFO Subareas 0 and 1) were managed bilaterally between Canada and Greenland as a single unit, while those further south along the Labrador and northeastern Newfoundland continental shelf (NAFO Subarea 2 and Divisions 3KL) were regulated by Canada within its 200-mile limit. The fishery for Greenland halibut beyond the 200-mile limit south of this area (NAFO Divisions 3LMNO) was unregulated. In 1995, based on the advice of the NAFO Scientific Council, the inshore area of northwestern Greenland (Division 1A) began to be regulated separately from the offshore areas, and all of the Greenland halibut south of the Davis Strait (NAFO Subareas 2 and 3) began to be regulated as a single unit under the management of the multinational NAFO Fisheries Commission. These changes were prompted by conservation concerns. For example, if the entire resource were a single biological unit, intense fishing effort in areas of localized abundance could cause significant damage to the resource as a whole. It is critical, therefore, that the stock structure in the Northwest Atlantic be elucidated as conclusively as possible.

Stock structure of Greenland Halibut in the Northeast Atlantic is less well understood. It has been assumed that a single stock resides in the waters around Iceland and the nearby Faeroe Islands (Sigurdsson 1981). Fish are found from the west side of the island around to the northern and southeastern coasts; the absence of Greenland halibut on the southern coast is associated with elevated water temperatures (Nizovtsev 1970). Spawning usually occurs in winter in the northwest, and along the continental slope of east Greenland at depths to 1000 m (Sigurdsson 1977). Juvenile (0-group) Greenland halibut have been caught from western Iceland to the southern tip of Greenland, with higher catches taken on the east Greenland continental shelf (Sigurdsson 1980). Postspawning fish undertake a spring feeding migration back to northern and eastern Iceland; by September the main feeding migration is over and they return west for spawning (Nizovtsev 1970). Research vessel surveys in 1992 and 1993 confirmed this pattern and suggest that the highest commercial densities are located to the west of Iceland (E. Hjörleifsson, Marine Research Institute, Reykjavik, Iceland, personal communication). The range of Greenland halibut in the Northeast Atlantic extends along the northwest shores of Spitsbergen northeast to the coast of Novaya Zemlya (Sorokin 1967) and along the entire White Sea

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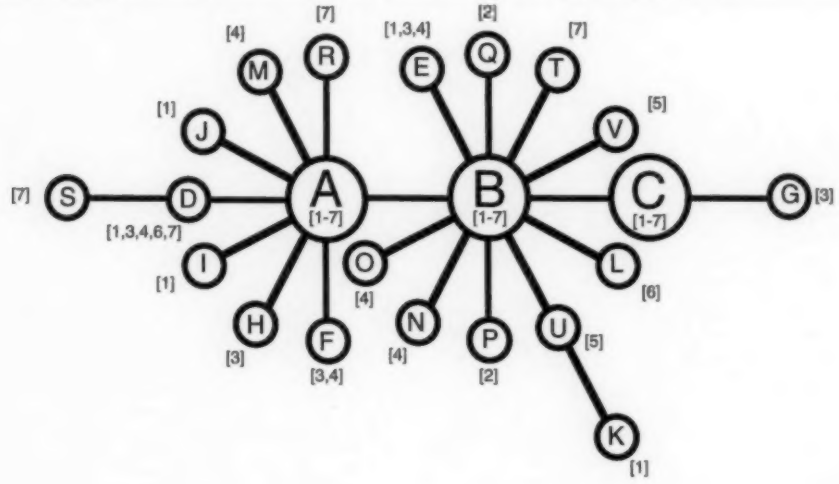
Fig. 2. Phylogenetic relationships of 22 genotypes among seven sample locations of Greenland halibut. A maximum parsimony network is shown; a single nucleotide substitution occurs along each network branch. Distributions of genotypes among regions are summarized from Table 1. Genotypes A, B, and C occur in all seven samples; the remaining genotypes occur in the samples indicated in brackets: [1] Northeast Slope; [2] Gulf of St. Lawrence; [3] Iceland; [4] Davis Strait; [5] Flemish Pass; [6] Norway; and [7] Northwest Greenland.



and Norwegian Atlantic coasts as far south as Bergen (Hognestad 1969). Recent research vessel surveys have captured Greenland halibut as deep as 1500 m off Norway (Anonymous 1996). The spawning area for Greenland halibut in the Northeast Atlantic may extend as far south as Traena Bank (66°N) and northward to southwestern Spitsbergen (Godo and Haug 1987).

A key component of the unit stock concept is that, to be treated as separate populations, fish stocks must be genetically as well as ecologically distinct (Ovenden 1990; Utter 1991; Dizon et al. 1992). Genetic differentiation in marine fish species has been examined by direct analysis of mitochondrial DNA (mtDNA) sequences, amplified in vitro by the polymerase chain reaction (PCR) (Carr and Marshall 1991*a*, 1991*b*; Bartlett and Davidson 1991; Pepin and Carr 1993; Carr et al. 1995). Here, we extend this analysis to Greenland halibut in the North Atlantic.

Materials and methods

Greenland halibut were collected by personnel of the Canadian Department of Fisheries and Oceans (DFO) and colleagues in Denmark (Greenland), Iceland, and Norway. Spawning aggregations of Greenland halibut are difficult or impossible to sample: known sites are usually ice-covered during spawning, and in the eastern Atlantic, spawning times and locations are largely unknown. We therefore choose a series of geographically isolated sites where Greenland halibut were known to be commercially abundant. Samples were collected from the northeastern Newfoundland continental shelf (hereinafter referred to as the Northeast Slope) (NAFO Division 3K), the estuary of the Gulf of St. Lawrence (Division 4S), Flemish Pass (Divisions 3LM), Davis Strait (Division 0B), northwestern Greenland at Jakobshavn fjord (Division 1A), western Iceland (65°16′N, 28°18′W), and Bear Island north of Norway (hereinafter referred to as Norway) (73°17′N, 14°47′W) (Fig. 1).

DNA was extracted from frozen muscle tissue as described by Carr and Marshall (1991*a*) or Bartlett and Davidson (1992). Forty fish were examined from each locality. A portion of the mitochondrial genome including a 401 base pair (bp) region at the 5′ end of the cytochrome *b* sequence was amplified by PCR, substantially as described by Carr and Marshall (1991*a*, 1991*b*). The oligonucleotides L14724, (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′; Irwin et al. 1991) and H15149 (5′-GCCCCTCAGAATGATATTTGTCCTCA-3′; Kocher et al. 1989) were used as primers for both amplification and sequencing. DNA sequencing was accomplished using fluorescent dye-terminator chemistry and an Applied Biosystems 373A automated DNA sequencer (Carr and Marshall 1991*b*). All sequences are given as their coding-strand equivalents.

Sequences were analysed and prepared for publication with the help of ESEE (version 3.00) (Cabot and Beckenbach 1989). Genetic heterogeneity within samples was estimated by the nucleon diversity (h) index for nonselfing populations and nucleotide diversity (π) index of Nei and Tajima (1981) as calculated by the Restriction Enzyme Analysis Package (REAP) (McElroy et al. 1991) from the pairwise haplotype divergences obtained using the Molecular Evolutionary Genetics Analysis (MEGA) program (Kumar et al. 1993). Genetic heterogeneity among samples was tested with the Monte Carlo χ^2 test of Roff and Bentzen (1989) from REAP; 5000 resamplings of the data matrix were used. The coancestry coefficient θ was calculated with the HAPLOID program (Weir 1990) to determine the proportion of genetic diversity attributable to subdivision among samples. The degree of gene flow among samples was estimated using the program of M. Slatkin (Department of Integrative Biology, University of California, Berkeley, CA 94729, U.S.A.). Pairwise Rogers' genetic distances (Rogers 1972) among populations were calculated by the NTSYS program of Rohlf (1992). Cluster analyses by the UPGMA and neighbor joining algorithms were performed with the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1993).

Results

Within the 401-bp region examined, 20 variable sites were identified among the 280 fish sampled (Fig. 2). All but two of the observed substitutions occur in the third codon position and would not result in an amino acid substitution. The other two substitutions were first position transitions that exchange valine for isoleucine. These variable sites define 22 genotypes

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Table 1. Distribution of mtDNA genotypes of Greenland halibut among seven sampling locations in the North Atlantic and adjacent waters.

		mtDNA genotype																				
Locality	*n*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Northeast slope	40	16	11	8	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gulf of St. Lawrence	40	12	18	5	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Iceland	40	17	14	7	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Davis Strait	40	18	10	4	2	1	1	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0
Flemish Pass	40	18	16	4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Norway	40	20	10	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0
Nortwestern Greenland	40	16	12	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
Totals	280	117	91	42	6	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	2	2	1

that differ by one to five nucleotide substitutions (Table 1). These sequences have been submitted to the Genome Sequence Data Base and assigned the accession numbers L77930–L77951. Genotype A is the most common genotype in all samples except that from the Gulf of St. Lawrence (overall mean 41.8%, range 30.0–50.0%); the most common alternative genotypes (B and C) also occur in all samples, at overall frequencies of 32.5% (range 25.0–45.0%) and 15.0% (range 10.0–22.5%), respectively. In the Gulf of St. Lawrence sample, genotype B is more abundant than A. Genotype D occurs in 2.5% of fish and in all samples except Flemish Cap and northwestern Greenland. The remaining genotypes typically occur only in one or two samples in one or two individual fish.

The Monte Carlo χ^2 test indicates no significant differences of genotype distributions among the seven samples ($\chi^2 = 138.03$, df = 126, $P > 0.10$). There are no significant differences if the Gulf of St. Lawrence sample is removed from the analysis ($\chi^2 = 98.06$, df = 90, $P > 0.15$) or if that sample is compared with a pool of the remaining samples ($\chi^2 = 25.91$, df = 42, $P > 0.26$). There are no significant differences if the western and eastern Atlantic populations are pooled separately and compared (Iceland and Norway versus all others, $\chi^2 = 17.20$, df = 16, $P > 0.35$). There are no significant differences if fish from the northern and southern extremes of the Northwest Atlantic are pooled and compared (Davis Strait and Northwest Greenland versus Northeast Slope and Flemish Pass, $\chi^2 = 11.88$, df = 14, $P > 0.75$). There are no significant differences if fish from within the 200-mile limit are pooled and compared with those outside the limit in the western Atlantic (Davis Strait and Northeast Slope versus Northwest Greenland and Flemish Pass, $\chi^2 = 19.08$, df = 14, $P > 0.068$).

The nucleon diversity (h) and nucleotide diversity (π) indices within samples are given in Table 2. The mean nucleon diversity is 0.69, which indicates that the probability that any two fish chosen at random will have different genotypes is about 70%. The Flemish Pass and adjacent Northeast Slope samples have the smallest and largest nucleon diversity, respectively. The nucleotide diversity calculation corrects the nucleon diversity index for the actual number of nucleotide substitutions between any two genotypes; for a 400-bp molecule, the mean value of 0.0025 indicates that any two fish chosen at random differ on average by about one nucleotide substitution in the gene region examined (i.e., 400 × 0.0025 = 1.0).

Nucleotide diversity and Rogers' distance between samples are given in Table 3. Calculation of the former includes a correction for nucleotide diversity within samples: a negative value indicates that the average within-sample variation is greater than the between-sample difference. This is the case for 13 of 21 pairwise comparisons. Rogers' distance describes the difference between populations as the pairwise Euclidean distance between haplotype frequencies. UPGMA cluster analysis will represent these differences among multiple samples on the assumption that genetic distance accumulates linearly with respect to time; neighbor joining analysis does not make this assumption. Both UPGMA (Fig. 3) and neighbor joining (Fig. 4) analyses of Rogers' distance indicate that such genetic differences as exist among Greenland halibut populations are not related to their geographic distribution. The relative distinctness of the Gulf of St. Lawrence sample reflects the reversal in relative frequency of the two most abundant genotypes. Otherwise, samples from opposite sides of the Atlantic are typically more similar than are geographically contiguous samples from the Northwest Atlantic.

Table 2. Haplotype (h) and nucleotide (π) diversity indices within samples of Greenland halibut from the North Atlantic and adjacent waters.

Population	Haplotype diversity	Nucleotide diversity
Northeast Slope	0.7304	0.002 799
Gulf of St. Lawrence	0.6975	0.002 516
Iceland	0.6734	0.002 391
Davis Strait	0.7266	0.002 969
Flemish Pass	0.6342	0.001 986
Norway	0.6759	0.002 558
Northwestern Greenland	0.7051	0.002 500
Mean	0.6919±0.000 16	0.002 531

The coancestry coefficient θ over the seven samples is -0.0033 ± 0.0072, that is, not significantly different from zero. This result also indicates that the proportion of genotypic differentiation attributable to subdivision among samples is negligible (Weir 1990). Slatkin and Maddison's (1989) estimate of $N_e m$ is based on a count of the minimum number of migration events (S) necessary to account for the observed distribution of genotypes among multiple populations. For example, the occurrence of a genotype in n samples requires a minimum of $S = n - 1$ events. From the phylogenetic relationships shown in Fig. 4 and the sample sizes and distribution of the 22 genotypes among the seven samples given in Table 1, S was calculated to be 26 (25 from the occurrence of genotypes in multiple populations, plus 1 from the distribution of genotype K with respect to genotype U), from which $N_e m$ was estimated as 0.8 (95% confidence interval 0.5–1.1).

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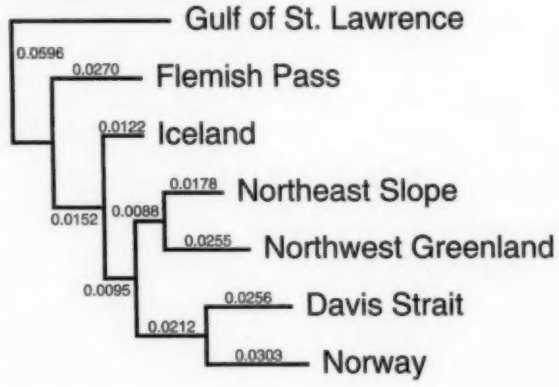
Table 3. Nucleotide divergences (below the diagonal) and Rogers' distances (above the diagonal) among samples of Greenland halibut from the North Atlantic and adjacent waters.

	Northeast slope	Gulf of St. Lawrence	Iceland	Davis Strait	Flemish Pass	Norway	Northwest Greenland
Northeast Slope	—	0.111 80	0.050 00	0.068 47	0.090 14	0.075 00	0.043 30
Gulf of St. Lawrence	–0.000 002	—	0.088 39	0.132 29	0.086 60	0.146 84	0.110 40
Iceland	–0.000 051	–0.000 028	—	0.075 00	0.053 03	0.075 00	0.050 00
Davis Strait	0.000 002	0.000 035	–0.000 007	—	0.088 39	0.055 90	0.088 39
Flemish Pass	–0.000 005	–0.000 027	–0.000 033	–0.000 009	—	0.088 39	0.090 14
Norway	0.000 004	0.000 057	–0.000 002	–0.000 038	0.000 003	—	0.086 60
Northwest Greenland	–0.000 033	–0.000 024	–0.000 036	0.000 060	0.000 000	0.000 062	—

Fig. 3. UPGMA cluster analysis of Rogers' distances among Greenland halibut populations.

Fig. 4. Neighbor-joining analysis of Rogers' distances among Greenland halibut populations. The network was rooted with the Gulf of St. Lawrence sample specified as the outgroup, as indicated by the UPGMA analysis in Fig. 3.



Discussion

Comparison of mtDNA sequence variation in Greenland halibut throughout the North Atlantic identifies extensive polymorphism (22 genotypes) and genetic diversity (global h = 0.69, global π = 0.0025). However, genotype proportions are not significantly differentiated among samples. Three major genotypes are present at approximately equal frequencies in all populations, except that the relative abundances of the two major genotypes are reversed in the Gulf of St. Lawrence sample (the difference is not statistically significant). There is no indication that such genetic variation as exists is subdivided among geographic samples: $\theta \simeq 0.0$, and cluster analysis does not identify any structure consistent with geographical stocks. Intermixing among Greenland halibut, between the northern and southern extremes of the commercial range in the western Atlantic, inside and outside the 200-mile Canadian economic zone, and between samples on either side of the Atlantic, appears to be sufficient to produce a single genetically homogeneous population in the North Atlantic.

Substantially identical results were obtained by Fairbairn (1981), who studied Greenland halibut populations in the Northwest Atlantic and Gulf of St. Lawrence with protein electrophoretic markers. She found that neither of two polymorphic loci was significantly differentiated among samples from Labrador to the Northeast Slope, and that the Gulf sample, although somewhat different in allele frequencies, was not significantly differentiable from the Atlantic samples. Fairbairn (1981) concluded that the Northwest Atlantic populations were a single interbreeding stock, whereas the Gulf population was a separate breeding area. (Fairbairn (1981) emphasized the presence of an essentially private allele at the *PHI1* locus in the Gulf sample. She pooled rare alleles for her χ^2 test and found no significant difference between the pool of Northwest Atlantic samples and the single Gulf sample. We re-analyzed her data by the Monte Carlo χ^2 test, which does not require pooling, and found this comparison to be significantly differentiated (χ^2 = 18.87, df = 4, $P < 0.003$).) Data from the cytoplasmic and nuclear genomes are thus in agreement as to the absence of significant stock structure in the Northwest Atlantic and the relative distinctiveness of the Gulf population.

The genetic structure of Greenland halibut differs markedly from that in Atlantic cod (*Gadus morhua*), which has been studied with the same genetic marker in the same general area of the North Atlantic (Carr and Marshall 1991*a*, 1991*b*; Pepin and Carr 1993; Carr et al. 1995; Crutcher 1996). Cod in the western Atlantic are also highly polymorphic (more than 40 genotypes), but all populations are dominated by a single genotype at high mean frequency. In contrast, cod in the eastern Atlantic show multiple alleles at moderate frequencies. The distribution of genotype proportions is uniform among populations in the western Atlantic, whereas populations on either side of the Atlantic are significantly differentiated (Carr and Marshall 1991*b*). Pairwise genetic distances among cod samples from Norway and the Barent's Sea are more than twice those of sample pairs drawn from anywhere in the

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commercial range of cod in the western Atlantic (Crutcher 1996). The same is true for Icelandic versus Norwegian cod (Árnason and Pálsson 1996). The pattern of reduced variation in the western North Atlantic has been interpreted as the result of bottleneck(s) subsequent to the colonization from the eastern North Atlantic, possibly during the most recent glaciation (Carr et al. 1995; cf. Cross and Payne 1978).

The differences in genetic patterns between the two species may be attributable to differing life-history characters. Greenland halibut, the more bathypelagic species, may have been less affected by glaciation if they remained at greater depths. As noted, they are commercially abundant to depths of 1800 m (Bowering and Brodie 1995) and have been caught as deep as 2200 m at western Greenland (Boje and Hareide 1993) and in the Flemish Pass (de Cardenas et al. 1996). Because the slope of the entire North Atlantic continental shelf is continuous at these depths (Fig. 1), the potential for extensive mixing exists along the slope contour. Atlantic cod, on the other hand, are generally confined to the more shallow banks of the continental shelf, which are separated by deep channels that this species is less likely to cross. As well, Greenland halibut larvae remain pelagic for an unusually long time and are more readily dispersed by current-driven drift (Templeman 1973) than are Atlantic cod. Tagging studies indicate that a substantial degree of mixing in Greenland halibut may result from long-distance movement of reproductive adults among populations, which is less extensive in cod (Lear 1984). It is known that Greenland halibut migrate between eastern Newfoundland waters and the Davis Strait area (Bowering 1984). Boje (1993) reported that fish tagged at southwestern Greenland have been recaptured at eastern Greenland, and Sigurdsson (1981) reported incidental recaptures of fish tagged in northern Iceland from as far east as the Faroe Islands and Bear Island and as far northeast as the Russian coast. The direction of movement may not be uniform: fish tagged off Newfoundland in southern areas have never been recaptured further north in the inshore areas of western Greenland (Bowering 1984), and there are no published reports of recaptures made west of the tagging sites in the eastern Atlantic. It must be remembered that a level of movement among populations that would be regarded as quite small from a tag-return perspective can lead to substantial gene flow and homogenization of allele frequencies. For haploid genetic markers such as mtDNA, the exchange of a single breeding female per pair of populations per generation is sufficient to prevent divergence of selectively neutral alleles (Slatkin 1985).

Based on meristic and morphometric characters, previous studies of stock structure in Greenland halibut have identified distinguishable groups of adult fish that are geographically localized. The present analysis suggests that such phenotypic differences likely reflect environmental influences rather than genotypic differences among isolated gene pools. Contrasting patterns of meristic or morphological versus genetic variation have been found in other marine teleost species (Leslie and Grant 1990; Roby et al. 1991; Pepin and Carr 1993). Spawning in marine fish species generally occurs over a broad geographic area, and extensive egg and larval drift across management unit boundaries may limit their genetic differentiation, despite differences in subsequent environmental histories that may affect phenotypic features (Sinclair 1988). Some degree of mixing through egg and larval flow is likely in Greenland halibut, particularly in areas such as the Gulf of St. Lawrence. Attempts to define "stocks" based on differences in meristic or morphological features must be supported by genetic evidence to confirm that the former differences reflect some degree of reproductive isolation rather than simply environmental distinctiveness (Pepin and Carr 1993). Studies of the distribution of local components of Greenland halibut for management purposes may find traditional methods helpful but must now recognize that extensive gene flow occurs among populations throughout the North Atlantic.

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Genetic variation and conservation of stream fishes: influence of ecology, life history, and water quality

Michael R. Heithaus and Roger H. Laushman

Abstract: Three stream-dwelling fish species were used to investigate effects of ecology, life history, and water quality on genetic variation. We sampled *Etheostoma caeruleum*, *E. blennioides*, and *Campostoma anomalum* from six streams of varying water quality. Allozyme electrophoresis revealed that the most ecologically specialized species, *E. caeruleum*, was the least variable ($P = 68.4\%$, $H_{obs} = 1.2\%$). *Etheostoma blennioides* was intermediate in specialization and variation ($P = 77.8\%$, $H_{obs} = 7.8\%$), and the least specialized species, *C. anomalum*, had the most variation ($P = 90.0\%$, $H_{obs} = 12.1\%$). This pattern conforms to Willis' niche-variation hypothesis and Selander and Kaufman's adaptation model. Differences in ecology, life history, and amount of genetic variation are responsible for differences in how variation is apportioned within and among populations and within and among rivers. Populations in the river with the worst water quality (Huron River) had the lowest within-population variation for each species; therefore, genetic variation may be a useful indicator of water quality. Lower genetic variation may result from selection associated with specific loci, e.g., *PGM-2*, in stoneroller minnows. However, indirect effects on population size probably contributed to the erosion of genetic variation. Ecology, life history, and pollution tolerance data combine as predictors of species' risk of genetic erosion.

Résumé : Trois espèces de poissons de cours d'eau ont été utilisées pour étudier les effets de l'écologie, du cycle vital et de la qualité de l'eau sur la variation génétique. Nous avons échantillonné *Etheostoma caeruleum*, *E. blennioides* et *Campostoma anomalum* dans six cours d'eau dont l'eau variait en qualité. L'électrophorèse des allozymes a révélé que l'espèce la plus spécialisée du point de vue écologique, *E. caeruleum*, était la moins variable ($P = 68{,}4\ \%$, $H_{obs} = 1{,}2\ \%$). *Etheostoma blennioides* occupait une place intermédiaire tant pour la spécialisation que pour la variation ($P = 77{,}8\ \%$, $H_{obs} = 7{,}8\ \%$), et l'espèce la moins spécialisée, *C. anomalum*, présentait la plus grande variation ($P = 90{,}0\ \%$, $H_{obs} = 12{,}1\ \%$). Ces résultats sont conformes à l'hypothèse de la variation des niches de Willis et aux modèles d'adaptation de Selander et Kaufman. Les différences du point de vue de l'écologie, du cycle vital et du degré de variation génétique sont responsables des différences en ce qui a trait à la répartition de la variation interpopulations et intrapopulation et inter- et intra-rivière. Les populations fréquentant la rivière dont l'eau était de la plus mauvaise qualité (rivière Huron) avaient la plus faible variation intrapopulation pour chacune des espèces ; par conséquent, la variation génétique pourrait être un indicateur utile de la qualité de l'eau. Une variation génétique plus faible peut résulter de la sélection liée à des loci spécifiques, p. ex. *PGM-2*, chez le roule-caillou. Toutefois, des effets indirects sur la taille de la population ont probablement contribué à l'érosion de la variation génétique. Les données sur l'écologie, le cycle vital et la tolérance à la pollution se combinent pour former des indicateurs du danger d'érosion génétique des espèces.
[Traduit par la Rédaction]

Introduction

Allozyme variation has been used extensively to estimate genetic variation within and among populations (Nevo et al. 1984; Hamrick and Godt 1990; Haglund et al. 1992). Species are often arranged into hierarchies of metapopulations, populations, and subpopulations with varied distributions of genetic variation within and among these levels of organization (Selander and Johnson 1973; Baumgartner 1985). Knowledge about patterns of genetic variation has become critical in attempts to preserve the genetic structure of species. Reduction in genetic variation is associated with reduced population growth rate (Leberg 1990), reduced fecundity (Quattro and Vrijenhoek 1989), and reduced ability to adapt to novel stress. Environmental stresses, e.g., pollution (Chagnon and Guttman 1989; Gillespie and Guttman 1989, 1993) and low population densities, are correlated with decreased genetic variation in both natural and experimental populations.

Ecology and life history are correlated with the amount and distribution of genetic variation (Willis 1973; Somero and Soulé 1974; Nevo et al. 1984; Zimmerman 1987; Hamrick and Godt 1990). Selander and Kaufman (1973) hypothesized that organisms experiencing the environment in a "coarse-grained" manner will have more genetic variation than organisms experiencing "fine-grained" environmental fluctuations. Willis' (1973) niche-variation hypothesis predicts that species in heterogeneous environments require broader tolerance ranges, and therefore maintain higher genetic variation, than species that inhabit homogeneous environments. The time-divergence hypothesis (Soulé 1972; Somero and Soulé 1974) predicts

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M.R. Heithaus[1] and R.H. Laushman. Department of Biology, Oberlin College, Oberlin, OH 44074, U.S.A.

[1] Author to whom all correspondence should be addressed. Present address: Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada. e-mail: mheithau@sfu.ca

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more genetic variation in species of temporally stable environments due to reduced directional selection.

Pollution has been linked to shifts in genotype frequencies of enzymes (Guttman 1994), e.g., phosphoglucomutase (PGM) and malate dehydrogenase (MDH), in *Campostoma anomalum* (stoneroller minnows; Gillespie and Guttman 1989), glucose-6-phosphate isomerase (GPI) in *Notropis spilopterus* (spotfin shiners; Gillespie and Guttman 1993), and differential survivorship of genotypes of isocitrate dehydrogenase (IDHP) and GPI in *Gambusia holbrooki* (eastern mosquitofish; Chagnon and Guttman 1989). Kcklak et al. (1994) showed that the progeny of *G. holbrooki* collected from a uranium-contaminated stream showed greater survivorship than progeny of fish from uncontaminated streams when exposed to uranium, suggesting a uranium-induced shift in genotype frequencies. Prolonged exposure to pollution could erode genetic variation at these and linked loci. Heavy-metal exposure and acid stress have been linked to lowered heterozygosity in populations of mosquitofish (Benton et al. 1994; Guttman 1994) and *Umbra limi* (central mudminnow; Kopp et al. 1992; Guttman 1994). Therefore, genetic variation may be used as an indicator of stress on fish populations (Guttman 1994; Fore et al. 1995*a*, 1995*b*). Reliable predictions regarding genetic structure and the risk of genetic erosion that are based on ecology, life history, and pollution tolerance would be of tremendous value to managers trying to protect natural populations.

Our purposes were to (*i*) determine overall levels of genetic variation in three species of stream fishes for the rivers studied; (*ii*) understand how the ecology and life history of these species relate to the overall level of genetic variation and geographic partitioning of this variation; (*iii*) assess the relationships among water quality, genetic variation, and population heterogeneity; and (*iv*) provide managers with a model to aid in the conservation of stream fishes.

Study species

Three fish species common in Ohio rivers were selected for study. These species have similar distributions throughout the midwestern and south-central United States (Trautman 1981). Stoneroller minnows (*Campostoma anomalum*) were the most abundant of the three species, followed by rainbow darters (*Etheostoma caeruleum*), and greenside darters (*E. blennioides*).

Etheostoma caeruleum (rainbow darter)

Etheostoma caeruleum has a very narrow habitat range. It is the most ecologically specialized species in this study, and is "moderately intolerant" to pollution (Bardarik 1965; Lotrich 1973; Trautman 1981; Ohio EPA 1989). *Etheostoma caeruleum* is found in swift current riffles with a sand, gravel, or rubble substrate and are rarely found in runs or pools between riffles (Winn 1958*a*, Schlosser and Toth 1984). Confined to riffles by dietary specialization (Turner 1921), *E. caeruleum* remains on one riffle throughout the summer. Low flow rates in late summer cause downstream migration into deeper riffles (Winn 1958*b*; Reed 1968; Schlosser and Toth 1984). Emigration and associated high mortality in young individuals, caused by increased risk of predation in pools, result in high temporal variation in population size (Schlosser and Toth 1984).

Etheostoma caeruleum feeds exclusively on small invertebrates (Turner 1921; Schlosser and Toth 1984). Common foods are mayfly, caddisfly, and midge larvae (Turner 1921; Adamson and Wissing 1977). Specialized diet may be why *E. caeruleum* is intensely affected by pollution. Lotrich (1973) found that *E. caeruleum* populations and other invertebrate-eating species were significantly reduced by strip-mine pollution. Reduction of *E. caeruleum* populations could have been a direct effect of the pollution, an indirect effect of prey reduction, or both.

Etheostoma blennioides (greenside darter)

Etheostoma blennioides is less specialized than *E. caeruleum* (Turner 1921; Bardarik 1965). They are "moderately intolerant" to pollution but more tolerant than *E. caeruleum* (Bardarik 1965; Ohio EPA 1989).

Etheostoma blennioides primarily inhabits deep, fast riffles, especially those with algae or moss-covered rubble (Fahy 1954; Winn 1958*a*, 1958*b*). However, younger individuals and occasionally adults are also found in shallow riffles and quiet waters (Fahy 1954; Trautman 1981). Males establish territories in deep, swift areas of riffles, under large, algae covered rocks, and exclude smaller males from these areas (Fahy 1954; Winn 1958*b*). Large females are evenly distributed throughout a riffle (Fahy 1954). *Etheostoma blennoides* often inhabits the same riffle throughout the breeding season and summer (Reed 1968) but is mobile outside the breeding season. *Etheostoma blennioides* migrates upstream just prior to the breeding season and can move long distances up semipermanent creeks (Schwartz 1965; Winn 1958*b*). *Etheostoma blennioides* moves downstream to larger riffles or quieter waters late in the season (Winn 1958*b*; Trautman 1981).

Etheostoma blennioides feeds primarily on invertebrates (Turner 1921; Fahy 1954), including midge larvae and mayfly larvae (Turner 1921). Fish remains and detritus have also been found in the stomachs of *E. blennioides* (Turner 1921), suggesting scavenging and a more flexible diet than *E. caeruleum* when faced with prey scarcity.

Campostoma anomalum (stoneroller minnow)

Campostoma anomalum has the widest niche of the fishes in this study as they inhabit all stream habitats. *Campostoma anomalum* is a generalist and "moderately tolerant" to pollution (Bardarik 1965; Lotrich 1973). *Campostoma anomalum* is common in all stream habitats and can dominate midwestern stream assemblages (Trautman 1981; Matthews et al. 1987). Site fidelity is variable (Miller 1962), as *C. anomalum* may move large distances up- and down-stream during a single day, within a general location (Matthews et al. 1987), or they may completely abandon an area if a large predator moves into a pool (Power et al. 1985). *Campostoma anomalum* feeds on algae and detritus in any stream habitat (Matthews et al. 1987; Gelwick and Matthews 1992). *Campostoma anomalum* is found in high relative abundances in polluted streams (Bardarik 1965; Lotrich 1973). A broad diet and feeding at a low trophic level may be partially responsible for *Campostoma anomalum*'s ability to survive in polluted waters.

Study sites

The rivers in this study represent the Lake Erie and Ohio River watersheds (Fig. 1). Beaver Creek and the Black, Huron,

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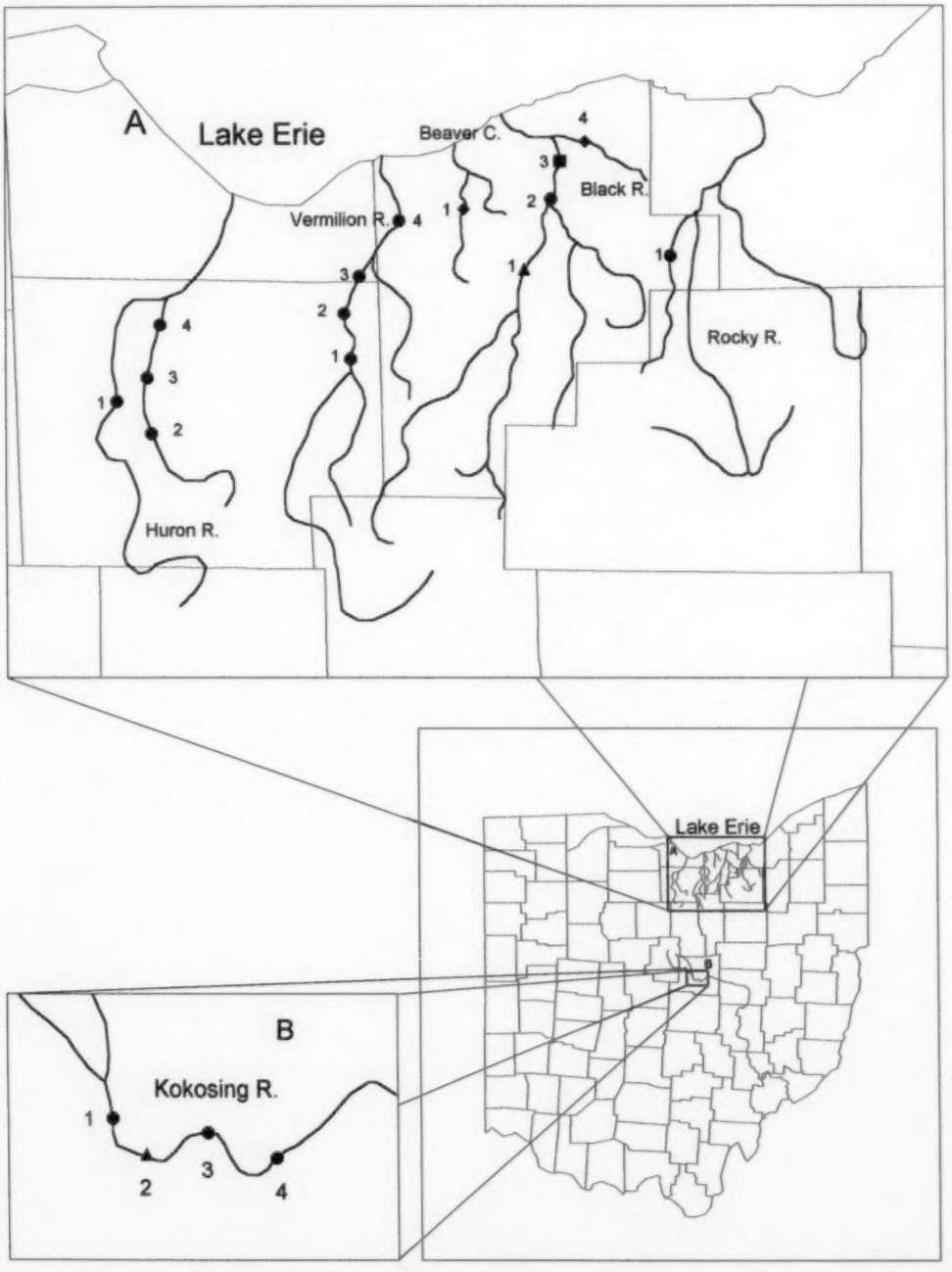
Fig. 1. Location of study populations in Ohio, USA. Beaver Creek and the Black, Huron, Rocky, and Vermilion rivers flow north into Lake Erie while the Kokosing flows southeast and eventually drains into the Ohio River. Twenty-four individuals of each species were collected at every site except where noted and at Black 2, where only six *Etheostoma caeuleum* were collected. ▲, only *E. caeruleum* collected; ◆, only *Campostoma anomalum* collected ($n = 8$ in Beaver Creek); ■, *E. blennioides* and *C. anomalum* collected.



Rocky, and Vermilion rivers drain north into Lake Erie. The Kokosing River joins the Mohican River to form the Walhonding River, which drains into the Ohio River. The Kokosing River is one of the cleanest in Ohio, and the Vermilion and upper Rocky River are relatively unpolluted (Ohio EPA 1993*a*, unpublished data). The Black River was heavily polluted in the early 1980s, but macroinvertebrate and fish communities indicated a significant improvement by 1992 (Ohio

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Table 1. Relative ranks of major pollutants in study rivers based on concentrations of inorganic, organic, and heavy metal contaminants.

River	Inorganic			Organic		Heavy metals
	Ca	Mg	Cl	NO_2–NO_3	PO_4	
Kokosing	1	1	1	1	1	1
Vermilion	2	2	2	2	2	2
Rocky	4	3	na	3	2	2
Black	3	4	3	4	4	4
Huron	5	5	4	6	5	5
Beaver Creek	na	na	"toxic levels"	5	6	5

Note: Rankings are based on Ohio EPA (unpublished data) and Ohio EPA (1993*a*, 1993*b*). 1, Least polluted; na, data not available.

EPA 1993*b*) Beaver Creek and the Huron River are heavily polluted (Ohio EPA 1993*b*, unpublished data). From this information, stream water quality can be generalized in the following order: Kokosing River > Vermilion River ≅ Rocky River > Black River > Beaver Creek ≅ Huron River (Table 1).

Materials and methods

Collections

Etheostoma blennoiodes, *E. caeruelum*, and *C. anomalum* were collected from Beaver Creek and the Black, Huron, Kokosing, Rocky, and Vermilion rivers from October to December 1994. Collections were made at four sites, separated by 3–4 river kilometers, in the Black, Huron, Kokosing, and Vermilion rivers (Fig. 1). One site each was collected in both Beaver Creek and Rocky River. At each site, 24 individuals of each species were collected. However, only *C. anomalum* ($n = 8$) were collected in Beaver Creek, and *E. caeruelum* were found and collected at only one site in the Black River ($n = 6$). *Etheostoma blennoides* was not found at site 4 on the Black River, and *C. anomalum* was absent at site 1. Although both *C. anomalum* and *E. caeruleum* were present at site 2 on the Kokosing River, only *E. blennoides* was collected. Voucher specimens are in the Lorain County Metro Parks collection at the Cleveland Museum of Natural History.

Electrophoresis

Muscle from the caudal peduncle was removed on the day of collection and stored at –80°C. Proteins were extracted by freezing the sample in liquid N_2 before crushing it into a fine powder. Several drops of 0.1 M Tris-HCl (pH = 7.0) were added to the powder to form a slurry. Miracloth® (Calbiochem) was placed on the slurry, and the liquid was absorbed onto Whatman 3MM chromatography wicks, which were stored at –80°C.

Starch gels were prepared with 10% starch (Sigma lot No. 54H0610) using the microwave technique described in Wendel and Weeden (1989). Each gel contained 24 experimental wicks and 1 standard wick (*Etheostoma caeruleum*). Gels resulted in 16 scoreable loci for *E. caeruleum* and 14 for *E. blennoides* (Table 2). *Campostoma anomalum* showed gene duplication and fixed heterozygosity at many loci (M.R. Heithaus and R.H. Laushman, in preparation[2]), so only the 10 loci showing diploid expression were used in this analysis. Loci were numbered sequentially beginning with the locus having the greatest anodal migration. Within loci, alleles were numbered in the same manner. Electrophoretic loci are used as independent estimates of genetic variation, thereby allowing comparisons among species based on different sets of loci (e.g., Nevo et al. 1984; Hamrick and Godt 1990).

Standard measures of within-population variation, including the mean number of alleles per locus (A), percent of loci polymorphic (P), observed heterozygosity (H_{obs}), and Hardy–Weinberg expected heterozygosity (H_{exp}) were obtained using BIOSYS-1 (Swofford and Selander 1981). Loci were considered polymorphic if any variation was detected. Variation among populations was summarized with Nei's (1978) estimate of genetic distance and contingency chi-square analysis of hierarchically arranged populations (watershed, river, population). Random-block ANOVA and paired t tests were used to test differences in heterozygosities among species and rivers (Archie 1985).

Table 2. Summary of enzymes and loci investigated.

Enzyme*	Buffer system	Locus	Species scored
Alcohol dehydrogenase	2	*ADH-1*	b, c
		ADH-2	a**
Esterase	7	*EST-1*	a, b, c
		EST-2	a, c
		EST-3	b
Glucose-6-phosphate isomerase	7	*GPI-1*	a, b, c
		GPI-2	a
Isocitrate dehydrogenase	TE	*IDHP-1*	a,** b, c
		IDHP-2	c
		IDHP-3	a
Lactate dehydrogenase	TE	*LDH-1*	a,** b, c
Malate dehydrogenase	9	*MDH-1*	a,** b, c
		MDH-2	b, c
		MDH-3	b, c
		MDH-4	b, c
Malic enzyme	TE	*MEP-1*	a, b, c
		MEP-2	a**
Mannose-6-phosphate isomerase	7	*MPI-1*	a,** b, c
		MPI-2	c
Phosphogluconate dehydrogenase	2	*PGDH-1*	a,** b, c
		PGDH-2	a
		PGDH-3	a
Phosphoglucomutase	2	*PGM-1*	a, b, c
		PGM-2	a

Note: All enzymes were extracted from muscle of the caudal peduncle. Buffer system numbers are from Soltis et al. (1983); buffer system TE is from Baumgartner (1986). a, *Campostoma anomalum*; b, *Etheostoma blennioidesc*; c, *E. caeruleum*.

*The staining procedures followed Solits et al. (1983) for glucose-6-phosphate isomerase and phosphoglucomutase; Wendel and Weeden (1989) for alcohol dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, malate-6-phosphate isomerase, and phosphogluconate dehydrogenase; Makert and Faulhaber (1965) for lactate dehydrogenase; and were modified from Soltis et al. (1983), with 10 mg alpha-napthyl proprionate substituted for alpha- and beta-napthyl acetate, for esterase.

**Locus was resolved but not included in analysis of *C. anomalum* because of gene duplication.

Results and discussion

Etheostoma caeruleum

Etheostoma caeruleum exhibited the lowest level of genetic variation in this study (random-block ANOVA, $p < 0.001$) (Table 3). *Etheostoma caeruleum* was polymorphic at 68.4% of the loci surveyed with an observed heterozygosity of 1.2%. The mean polymorphism per population was highest in the Kokosing River, followed, in order of decreasing polymorphisms, by the Rocky, Vermilion, Huron, and Black rivers.

[2] Heithaus, M.R., and Laushman, R.H. Genetic variation in benthic stream fishes: gene duplication in *Campostoma anomalum*.

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Table 3. Genetic variation within species and river systems.

River	*R*	*N*			*A*			*P*			H_{obs}		
		Ec	Eb	Ca	Ec	Eb	Ca	Ec	Eb	Ca	Ec	Eb	Ca
Species overall	—	295	381	351	1.8	2.1	2.4	68.4	77.8	90.0	0.012 (0.005)	0.078 (0.038)	0.121 (0.040)
Huron	5	96	95	96	1.2	1.3	1.3	15.6	19.6	25.0	0.009 (0.005)	0.016 (0.012)	0.012 (0.005)
Beaver	5	0	0	8	—	—	1.3	—	—	30.0	—	—	0.063 (0.034)
Black	4	7	72	57	1.1	1.5	1.7	6.3	33.4	46.6	0.009 (0.009)	0.118 (0.063)	0.191 (0.073)
Rocky	2	24	24	24	1.3	1.4	2.0	18.8	28.6	70.0	0.008 (0.004)	0.045 (0.022)	0.225 (0.072)
Vermilion	2	96	96	96	1.3	1.4	2.0	17.2	28.6	60.0	0.014 (0.008)	0.055 (0.026)	0.178 (0.058)
Kokosing	1	72	94	70	1.2	1.5	1.8	20.9	34.0	60.0	0.016 (0.008)	0.128 (0.068)	0.128 (0.046)

Note: Values for species-level variation are derived from pooled data. Within-river values are averages of populations weighted by sample size within rivers. Values in parentheses are SE. *R*, river water quality rank (1, high; 5, poor; see Table 1); *N*, number of individuals sampled; *A*, mean number of alleles per locus; *P*, mean percent of loci polymorphic; H_{obs}, observed heterozygosity; Ec, *Etheostoma caeruleum*; Eb, *E. blennioides*; Ca, *Campostoma anomalum*.

Populations ranged from $P = 6.3\%$ to 25.0%. (Table 4). There was no significant difference in observed heterozygosity among rivers (ANOVA, $p = 0.856$).

Six of 35 (17.1%) polymorphic loci over all populations deviated significantly from Hardy–Weinberg equilibrium ($p < 0.01$). The populations that were out of equilibrium contained one common allele and one or two rare alleles. Three populations contained a single homozygote for a rare allele while the other three had one homozygote for the rare allele and one heterozygote. Therefore, a real deviation from Hardy–Weinberg equilibrium is unlikely in the natural populations.

Nearly all of the differences in genetic composition among populations is explained by differentiation between the Ohio River and Lake Erie watersheds (Fig. 2). Nearly all of this differentiation is due to the fixation of *MPI-1* for different alleles, and different common alleles at the *GPI-1* locus (Appendix 1). Populations within the Lake Erie watershed were heterogeneous (Table 5), with most of this variation explained by the *MEP-1* locus and unique alleles at *LDH-1* in Vermilion River populations. Populations in both the Huron River and Kokosing River were homogeneous (Table 5). Despite heterogeneity within the Lake Erie watershed, populations showed very little genetic divergence as measured by Nei's (1978) genetic distance (Fig. 2).

Genetic variation in *E. caeruleum* is related to the absolute and relative population abundances found in Ohio EPA surveys. Historically, *E. caeruleum* have been found in the Black River (Trautman 1981), but they were not found in 1982 or 1992 Ohio EPA surveys. The Ohio EPA collected small numbers in the Rocky ($n = 6$) and Huron rivers ($n = 24$) (Ohio EPA unpublished data). These rivers exhibited the lowest levels of genetic variation. The Kokosing and Vermilion rivers had much larger populations ($n = 267$ and $n = 566$ respectively, Ohio EPA unpublished data) and almost double the observed heterozygosity of the other rivers. Kokosing River populations were more genetically variable than those in the Vermilion River. It should be noted that EPA sampling methods are biased against collection of small, benthic riffle species (e.g., darters). Therefore, collection sizes cited above should be considered as a relative measure of population sizes. Data collected during this study supported the relative ranks of population sizes but indicated much larger absolute population sizes. For example, we obtained genetic samples from 96 *E. caeruleum* in the Huron River, and collected more than twice as many overall, compared with 24 collected by EPA samples. Also, a recent survey of approximately 15 river kilometres of the Vermilion River yielded over 800 *E. caeruleum* (M.R. Heithaus and C. Grame, in preparation[3]).

Table 4. Variation within and among *Etheostoma caeruleum* populations.

Population	*N*	*A*	*P*	H_{obs}	H_{exp}
Black 2	7	1.1	6.3	0.009 (0.009)	0.009 (0.009)
Huron 1	24	1.3	25.0	0.008 (0.004)	0.013 (0.006)
Huron 2	24	1.2	18.8	0.021 (0.016)	0.019 (0.014)
Huron 3	24	1.1	12.5	0.005 (0.004)	0.010 (0.008)
Huron 4	24	1.1	6.3	0.003 (0.003)	0.003 (0.003)
Kokosing 1	24	1.2	18.8	0.013 (0.008)	0.013 (0.013)
Kokosing 3	24	1.2	18.8	0.016 (0.009)	0.015 (0.009)
Kokosing 4	24	1.3	25.0	0.018 (0.009)	0.022 (0.013)
Rocky 1	24	1.3	18.8	0.008 (0.004)	0.013 (0.008)
Vermilion 1	24	1.1	25.0	0.029 (0.021)	0.035 (0.019)
Vermilion 2	24	1.3	12.5	0.003 (0.003)	0.008 (0.006)
Vermilion 3	24	1.1	25.0	0.023 (0.013)	0.027 (0.015)
Vermilion 4	24	1.1	6.3	0.003 (0.003)	0.003 (0.003)

Note: Values in parentheses are SE. *N*, number of individuals sampled; *A*, mean number of alleles per locus; *P*, percent of loci polymorphic; H_{obs}, observed heterozygosity; H_{exp}, Hardy–Weinberg expected heterozygosity.

Low genetic variation in *E. caeruleum* appears to be correlated with water quality. The heavily polluted Huron River had the lowest average polymorphism and heterozygosity of the rivers with multiple populations. In contrast, the populations in the relatively unpolluted Kokosing River had the highest levels of average polymorphism and heterozygosity. However, observed heterozygosity in the Kokosing River was not significantly higher than in the Huron River (*t* test, $p = 0.344$). The failure to achieve statistical significance is probably due to the low level of heterozygosity in the species, but we believe the trend is biologically significant. Exposure to pollution can cause changes in genotype frequencies (Gillespie and Guttman 1989, 1993) through differential survival of genotypes (Chagnon and Guttman 1989; Kcklak et al. 1994), which could result in loss of genetic variation. Pollution also could be indirectly responsible for the reduced variation by decreasing invertebrate prey populations, thereby causing a collapse in *E. caeruleum* populations. Drift could subsequently erode genetic variation. A long-term study would be required to estimate the effect of water quality on *E. caeruleum* genetic variation.

[3] Heithaus, M.R., and Grame, C. Fish communities of the Vermillion River Watershed.

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Fig. 2. Nei's (1978) genetic distance among *Etheostoma caeruleum* populations. Note the small genetic differentiation among populations within watersheds. The large differentiation between the Ohio River watershed and Lake Erie watersheds is almost entirely due to differentiation at *GPI-1* and *MPI-1*.

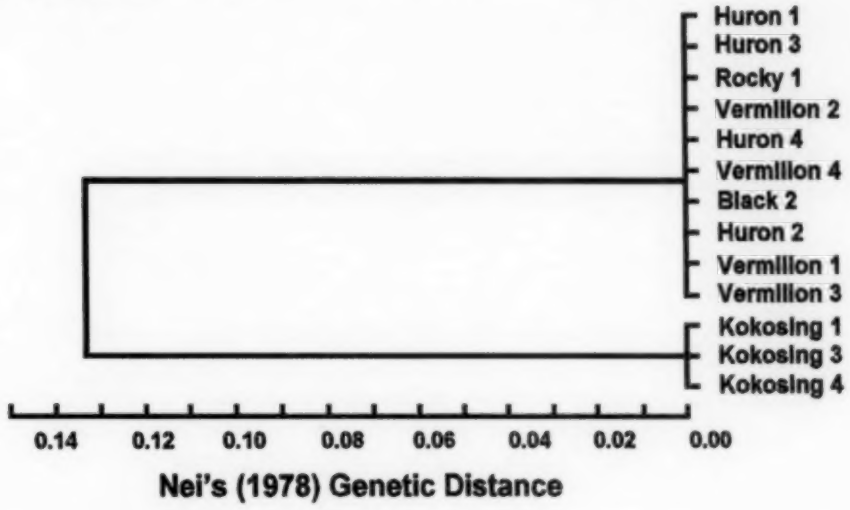

Table 5. *P* values for contingency chi-square tests for population heterogeneity within rivers and watersheds.

	Species		
River	*E. caeruleum*	*E. blennioides*	*C. anomalum*
Entire sample	<0.000 01	<0.000 01	<0.000 01
Lake Erie watershed	0.030 47	<0.000 01	<0.000 01
Black River		0.000 07	<0.000 01
Huron River	0.362 34	0.370 44	0.130 72
Vermilion River	0.032 33	<0.000 01	0.060 67
Ohio River watershed			
Kokosing River	0.377 37	0.000 21	0.000 01

Note: Values for the Kokosing River are equal to values for the Ohio River watershed. Populations in the Rocky River (all species), Black River (*E. caeuleum*), and Beaver Creek (*C. anomalum*) are included in calculations for heterogeneity within the Lake Erie watershed. Populations within the Huron River are homogeneous for all three species. Only the Vermilion River contains heterogeneous populations of *E. caeruleum* which is primarily due to the *MEP-1* locus. This locus in Vermilion River populations and rare alleles at *LDH-1* in Vermilion River populations are the primary cause of heterogeneity in *E. caeruleum* populations in the Lake Erie watershed. A complete data set for locus by locus chi-square tests is available upon request.

Table 6. Variation within and among *Etheostoma blennioides* populations.

Population	*N*	*A*	*P*	H_{obs}	H_{exp}
Black 1	24	1.5	28.6	0.098 (0.054)	0.094 (0.045)
Black 2	24	1.5	28.6	0.119 (0.067)	0.091 (0.048)
Black 3	24	1.6	28.6	0.137 (0.069)	0.117 (0.053)
Huron 1	24	1.2	14.3	0.012 (0.009)	0.012 (0.009)
Huron 2	24	1.2	14.3	0.024 (0.017)	0.023 (0.016)
Huron 3	23	1.4	28.6	0.009 (0.007)	0.021 (0.010)
Huron 4	24	1.3	21.4	0.018 (0.015)	0.023 (0.015)
Kokosing 1	24	1.6	42.9	0.128 (0.073)	0.097 (0.047)
Kokosing 2	22	1.4	28.6	0.136 (0.075)	0.105 (0.055)
Kokosing 3	24	1.4	28.6	0.137 (0.075)	0.107 (0.052)
Kokosing 4	24	1.6	35.7	0.158 (0.084)	0.177 (0.058)
Rocky 1	24	1.4	28.6	0.045 (0.022)	0.042 (0.021)
Vermilion 1	24	1.4	28.6	0.065 (0.045)	0.053 (0.034)
Vermilion 2	24	1.4	28.6	0.030 (0.019)	0.039 (0.019)
Vermilion 3	24	1.5	35.7	0.086 (0.047)	0.077 (0.041)
Vermilion 4	24	1.3	21.4	0.039 (0.025)	0.035 (0.022)

Note: Values in parentheses are SE. *N*, number of individuals sampled; *A*, mean number of alleles per locus; *P*, percent of loci polymorphic; H_{obs}, observed heterozygosity; H_{exp}, Hardy–Weinberg expected heterozygosity.

Etheostoma caeruleum was found at only one of four sites sampled in the Black River, and only in very low abundance at that site. This population had the lowest level of polymorphism and one of the lowest levels of heterozygosity (Table 4). This pattern is probably a result of historical rather than ongoing processes, as the Black River was highly polluted in the early 1980s. No *E. caeruleum* was found by the Ohio EPA in 1982 and 1992 surveys. The absence of *E. caeruleum* in the Black River during these surveys suggests a severe population bottleneck induced by pollution or a possible founder event following water quality improvement.

In summary, genetic patterns in *E. caeruleum* appear to be most influenced by river pollution and population size. However, historical patterns leading to drift may be important in rivers supporting small populations (e.g., Black River).

Etheostoma blennoides

Etheostoma blennoides were polymorphic at 77.8% of surveyed loci and had 7.8% observed heterozygosity, which is intermediate between *E. caeruleum* and *C. anomalum* (Table 3). Polymorphism within rivers and populations was higher than reported values for other freshwater fish, whereas heterozygosity was comparable with other studies (see Nevo et al. (1984) for a summary of genetic variation in other fish species).

Mean population polymorphism per river was highest in the

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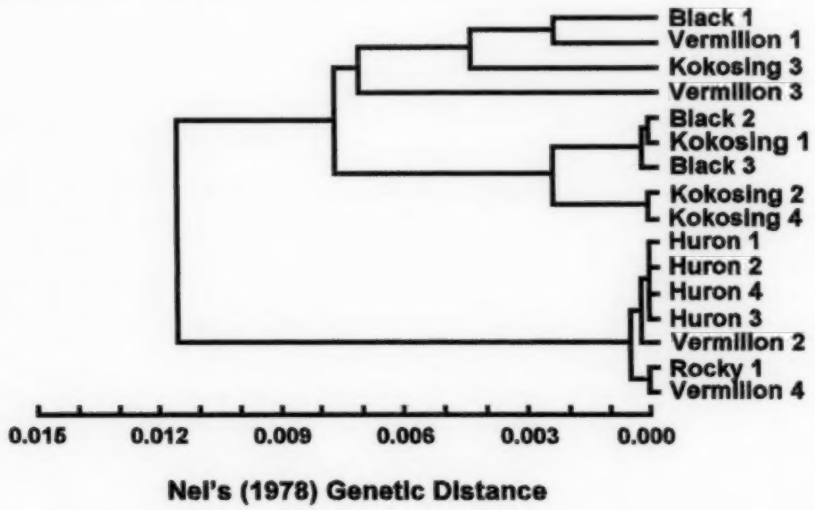
Fig. 3. Nei's (1978) genetic distance among *Etheostoma blennioides* populations. Note that populations do not cluster respective to watershed or river system except on the polluted Huron River, which has homogeneous populations.



Kokosing and Black rivers and lowest in the Huron River (Table 3). Populations varied from $P = 14.3\%$ to 42.9% (Table 6). Mean heterozygosity showed the same trend as polymorphism (Table 6) even though heterozygosity among rivers was not statistically different (random block ANOVA, $p = 0.255$). However, paired t tests (Archie 1985) of rivers with multiple populations revealed a slight trend towards lower heterozygosity in the Huron River. The P values for comparisons of Vermilion River – Kokosing River, Vermilion River – Black River, and Kokosing River– Black River were relatively high ($P = 0.139$, 0.269, and 0.401, respectively) in relation to comparisons of Huron River – Vermilion River, Huron River – Kokosing River, and Huron River – Black River ($P = 0.066$, 0.111, and 0.111, respectively). Observed heterozygosity was greater than expected in 12 of the 16 populations (sign test, $p < 0.05$). Three of the four populations in which $H_{obs} \leq H_{exp}$ were in the heavily polluted Huron River (Table 6).

Etheostoma blennoides deviated from Hardy–Weinberg equilibrium for 14 of 64 (21.9%) polymorphic loci over all populations ($p < 0.05$). Six of the deviant loci were due to rare alleles, with five containing a single homozygote for the rare allele and one with a homozygote and a heterozygote. Of the remaining eight deviant loci, six occured at the *MEP-1* locus in Kokosing River (four) and Black River (two) populations.

The majority of genetic variation in *E. blennioides* is due to variation among populations irrespective of river system. Populations in the less polluted rivers were heterogeneous (Table 5), and Nei's genetic distances for populations did not assort by river system but instead clustered populations from different river systems (Fig. 3). Huron River populations clustered together and were homogeneous. The Black and Kokosing river populations were the most variable and most genetically similar to each other. These rivers contained the largest populations during this study. Despite pollution in the Black River, *E. blennoides* remained in high abundance and underwent population growth (Ohio EPA, unpublished data). *Etheostoma blennoides* populations were not likely subjected to a bottleneck as was *E. caeruleum* and thus maintained high levels of genetic variation. Echelle et al. (1976) found that the presence of congeneric competitors led to lower genetic variation in two darter species. It is possible that the crash of *E. caeruleum* populations in the Black River allowed *E. blennoides* populations to maintain greater genetic variation than populations where competitor species are present.

Huron River populations were the least variable within and among populations (Fig. 3). Small population size is one possible explanation for lower genetic variation. Pollution also may be eroding genetic variation directly through selection or indirectly by reducing population size.

Genetic patterns in *E. blennoides* appear to be influenced by intense pollution and population size. Intermediate levels of pollution do not appear to have an effect on genetic variation, as *E. blennoides* populations can remain large under moderately polluted conditions.

Campostoma anomalum

Campostoma anomalum was the most genetically variable species in the study with 90.0% polymorphism and 12.1% observed heterozygosity. Mean polymorphism within rivers was high in the Rocky, Vermilion, and Kokosing rivers and low in the Huron River (Table 3). Populations ranged from $P = 10.0\%$ to 70.0% (Table 7). Observed heterozygosity was 12.1%, which was not significantly different from $H_{exp} = 14.7\%$. Mean heterozygosity was greatest in the Rocky and Black rivers and lowest in the Huron River (Table 3).

Twelve of 74 loci (16.2%) were out of Hardy–Weinberg equilibrium. Only one of these was due to a rare allele. Eight of ten populations were out of equilibrium at the *EST-1* locus owing to heterozygote deficiency ($F_{IS} = 0.405$).

Genetic differentiation in *C. anomalum* occurred primarily at the among-river level. Populations within less polluted rivers were significantly heterogeneous, with the exception of the Vermilion River, which was heterogeneous at the $p < 0.10$

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Table 7. Variation within and among *Campostoma anomalum* populations.

Population	*N*	*A*	*P*	H_{obs}	H_{exp}
Black 2	24	1.7	40.0	0.158 (0.069)	0.223 (0.091)
Black 3	9	1.9	60.0	0.251 (0.090)	0.273 (0.090)
Black 4	24	1.6	40.0	0.165 (0.079)	0.179 (0.086)
Huron 1	24	1.3	30.0	0.042 (0.025)	0.039 (0.023)
Huron 2	24	1.4	30.0	0.029 (0.015)	0.028 (0.015)
Huron 3	24	1.3	30.0	0.029 (0.018)	0.028 (0.017)
Huron 4	24	1.1	10.0	0.017 (0.017)	0.016 (0.016)
Kokosing 1	24	1.8	60.0	0.143 (0.055)	0.162 (0.064)
Kokosing 3	22	1.8	60.0	0.082 (0.032)	0.110 (0.059)
Kokosing 4	24	1.7	60.0	0.160 (0.056)	0.179 (0.072)
Rocky 1	24	2.0	70.0	0.225 (0.072)	0.230 (0.076)
Vermilion 1	24	2.1	60.0	0.150 (0.047)	0.205 (0.077)
Vermilion 2	23	2.0	60.0	0.138 (0.054)	0.189 (0.079)
Vermilion 3	24	1.9	60.0	0.213 (0.075)	0.221 (0.083)
Vermilion 4	24	2.0	60.0	0.212 (0.077)	0.230 (0.082)
Beaver Creek	8	1.3	30.0	0.063 (0.034)	0.059 (0.032)

Note: Values in parentheses are SE. *N*, number of individuals sampled; *A*, mean number of alleles per locus; *P*, percent of loci polymorphic; H_{obs}, observed heterozygosity; H_{exp}, Hardy–Weinberg expected heterozygosity.

level (Table 5). As with both darter species, Huron River populations were homogeneous. Populations within rivers were more similar to each other than to populations in other rivers (Fig. 4).

The Vermilion and Kokosing rivers had the largest populations (Ohio EPA, unpublished data) and the highest polymorphisms for rivers with multiple populations. The Black River population was smaller than the Vermilion and Kokosing rivers (Ohio EPA, unpublished data), but heterozygosity was not significantly different for these populations (*t* tests, $p = 0.965$ for Black River – Vermilion River, and $p = 0.452$ for Black River – Kokosing River) (Table 7). The Huron River contained only slightly smaller populations than the Black River (Ohio EPA unpublished data, personal observation), but had lower genetic variation than the other three rivers with multiple populations (*t* tests, $p = 0.069$ for Huron River – Black River, $p = 0.059$ for Huron River – Kokosing River, and $p = 0.022$ for Huron River – Vermilion River). This pattern suggests that pollution is more important than population size in the erosion of genetic variation in *C. anomalum*

Genotypic ratios of the *PGM-2* locus in *C. anomalum* reflect water quality (Gillespie and Guttman 1989). *Campostoma anomalum* in southwestern Ohio showed a decrease in the **a***a* and **a***b* genotypes below pollution influx. A similar pattern emerged in this study (Fig. 5). In the relatively clean Kokosing and Vermilion rivers the pollution-sensitive **1*1* (= **a***a*) and **1*2* (= **a***b*) genotypes accounted for 100% and 97.9% of individuals, respectively, and 91.6% in the Rocky River. The rivers with poorer water quality showed a reduction in frequency of the sensitive genotypes. In the Black River, 69.6% of individuals contained sensitive genotypes. The **1* (= **a*) allele only existed in 2 heterozygotes out of 96 individuals in the polluted Huron River and was completely absent in Beaver Creek, which is also polluted and may contain "toxic levels" of chloride (Table 1).

Campostoma anomalum has extremely high species-level variation and large populations in all streams. Pollution is correlated with erosion of genetic variation within rivers, and allele frequencies at the *PGM-2* locus provide a good indicator of water quality.

Ecology, life history, and genetic variation

River fishes show varying patterns of genetic variation within and among populations. Zimmerman (1987) suggested that benthic species had lower genetic variation and greater population heterogeneity than midwater species. *Etheostoma caeruleum*, *E. blennoides*, and *C. anomalum* are all benthic species, yet have greater polymorphism than some midwater species in Zimmerman's (1987) study. The trends Zimmerman (1987) observed may have been biased by the particular benthic species studied. Many were solitary species with low population densities within riffles, unlike the benthic species in this study. We propose that the distinction between benthic and midwater species is a false dichotomy when attempting to explain patterns of genetic variation within and among populations of stream fishes.

Contrary to Zimmerman's (1987) predictions for a benthic form, *E. caeruleum* populations are homogeneous within most rivers (Table 5). *Etheostoma blennoides* and *C. anomalum* conform to the expectation of heterogeneity in most rivers but show homogeneity in the Huron River. Also, the species in this study show a trend of decreasing variation with decreasing niche width. These results suggest vertical habitat alone is not a major determinant of genetic variation. The niche-variation hypothesis (Willis 1973; Nevo et al. 1994) and adaptation strategy of Selander and Kaufman (1973) explain the level of genetic variation within species. Population heterogeneity is determined by both the level of variation in a species and dispersal (gene flow).

Etheostoma caeruleum is the most specialized species, inhabiting a narrow habitat and feeding niche, which is correlated with less genetic variation. Also, individuals would not experience large differences in environmental factors and would be subjected to the same average environmental conditions. The apparent low level of gene flow in this species would predict heterogeneity and appreciable genetic differentiation among populations; however insufficient genetic variation exists at the species level for differentiation of populations.

Etheostoma blennoides is intermediate in specialization and genetic variation. Because *E. blennoides* can inhabit discrete habitats (e.g., riffles, runs, pools), they experience environmental factors in a more "coarse-grained" manner than *E. caeruleum* and have a wider niche. Maintenance of higher genetic variation is predicted. Gene flow between populations does not appear to be significant. The combination of high variation and low gene flow allows populations within a single river to diverge such that populations cluster irrespective of river system.

Campostoma anomalum, the most generalized species in this study, is also the most genetically variable. *Campostoma anomalum* is found in all river environments and thus has a wide niche. The species also inhabits discrete habitats. *Campostoma anomalum* should use a more "coarse-grained" approach than the other two species and, therefore, maintain the highest level of variation. Restricted gene flow leads to increased differentiation among populations.

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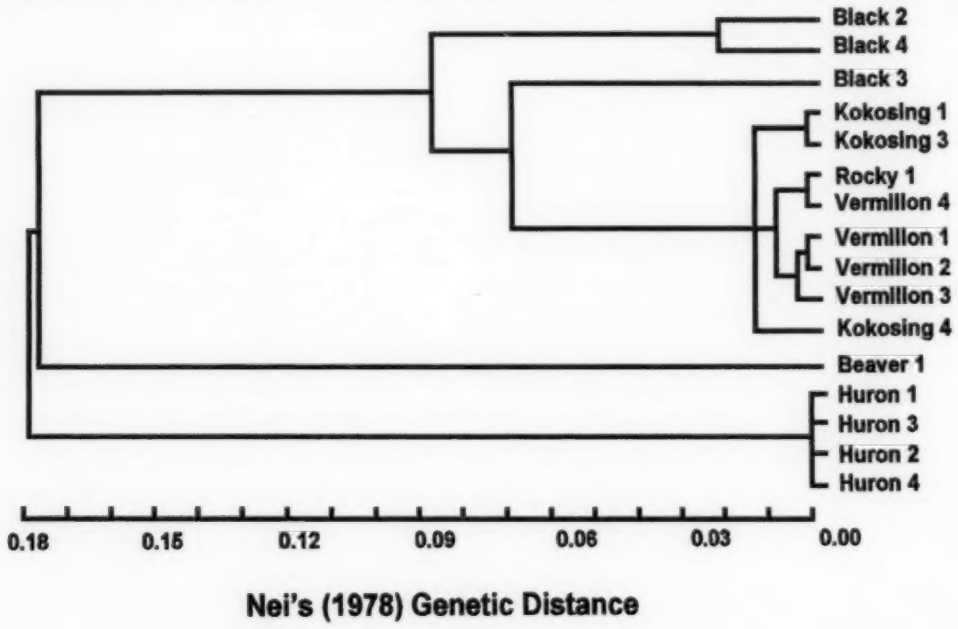
Fig. 4. Nei's (1978) genetic distance among *Campostoma anomalum* populations. There is no differentiation between watersheds but populations cluster by river system. Note that the polluted Huron River has homogeneous populations with almost no genetic divergence.



Niche breadth and interpopulation dispersal may be primary determinants of genetic variation and population differentiation. However, there are many potentially confounding variables. Physical factors, such as channel terracing, amount of suitable habitat, or large distance between suitable habitats, could lower variation within populations and increase variation between populations. Biotic factors could affect heterogeneity or genetic variation through competition (Echelle et al. 1976). Human-generated disturbances could lower population size (reduce variation) or inhibit dispersal (increase heterogeneity). All of these factors must be considered in making predictions about the partitioning of genetic variation.

Water quality and genetic variation

Overall genetic variation within and among rivers, and genotype frequencies at specific loci, are evidently good indicators of stream water quality. In the polluted Huron River, *E. blennoides* and *C. anomalum* populations had the lowest genetic variation, and *E. caeruleum* populations had the lowest genetic variation of multiple-population rivers. Also, Huron River populations were homogeneous for all three species, which could be a result of similar selection pressures, such as pollution, throughout the river. The predictions based on these genetic data parallel those of the Ohio EPA (1993*a*, 1993*b*, unpublished data), which were for the highest genetic variation in the Kokosing and Vermilion rivers and the lowest genetic variation in the Huron River. Differences in water quality could explain the significantly lower variation in the Huron River than the Black River despite similar relative abundances of each species. Direct effects of pollution and (or) indirect effects of low population density in the Huron River have probably reduced genetic variation in all three species.

As suggested by previous authors (Guttman 1994; Fore et al. 1995*a*, 1995*b*), we believe that patters of genetic variation can be used as an indicator of population stress. Although we have not determined the contaminants that are likely to be responsible for the erosion of variation, we have shown that populations inhabiting streams of lower water quality generally show decreased heterozygosity and polymorphism and are more homogeneous. Therefore, heterozygosity and the percent of polymorphic loci could be simple indicators of water quality and stress in stream fish populations, if comparative data are available (e.g., control samples from unstressed populations).

This study and the work of Gillespie and Guttman (1989) shows that the *PGM-2* locus in *C. anomalum* could potentially be used as a tool for rapid assessment of water quality. A sample of 24 *C. anomalum* per site from different areas of a stream should yield sufficient data to make inferences about stream water quality. This technique could also be used over a small scale, revealing the effects of land use patterns and discharges and the size of recovery zones.

We feel that using the *PGM-2* locus in *C. anomalum* as a bioindicator is more effective than monitoring water chemistry. Unlike water chemistry studies, the *PGM-2* locus shows the biological consequences of pollution. This method is also more likely to reflect the effects of pulsed pollutants in a system. Spot checks of water chemistry might miss nonpoint pulses of pollutants. However, the effects of such pulsed discharges would be observed in the genotype frequencies of *C. anomalum* after a pollution event as a result of mortality or dispersal of resident fish. Also, electrophoretic sampling is more cost effective than running a continuous water chemistry monitoring program ($30 000/year for each site). Electrophoretic sampling is also able to cover a larger area of a river.

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Fig. 5. Proportions of pollution sensitive (*1*1 and *1*2) and insensitive (*2*2) PGM-2 phenotypes of *Campostoma anomalum* within rivers. The relatively unpolluted Kokosing and Vermilion rivers show very high proportions of the sensitive phenotypes while the polluted Huron River has a high proportion of the insensitive phenotype. Beaver Creek, which is also highly polluted and may contain toxic levels of chloride, contains no individuals with a sensitive genotype. The Black River has an intermediate level of both phenotypes, which is consistent with its intermediate water quality.

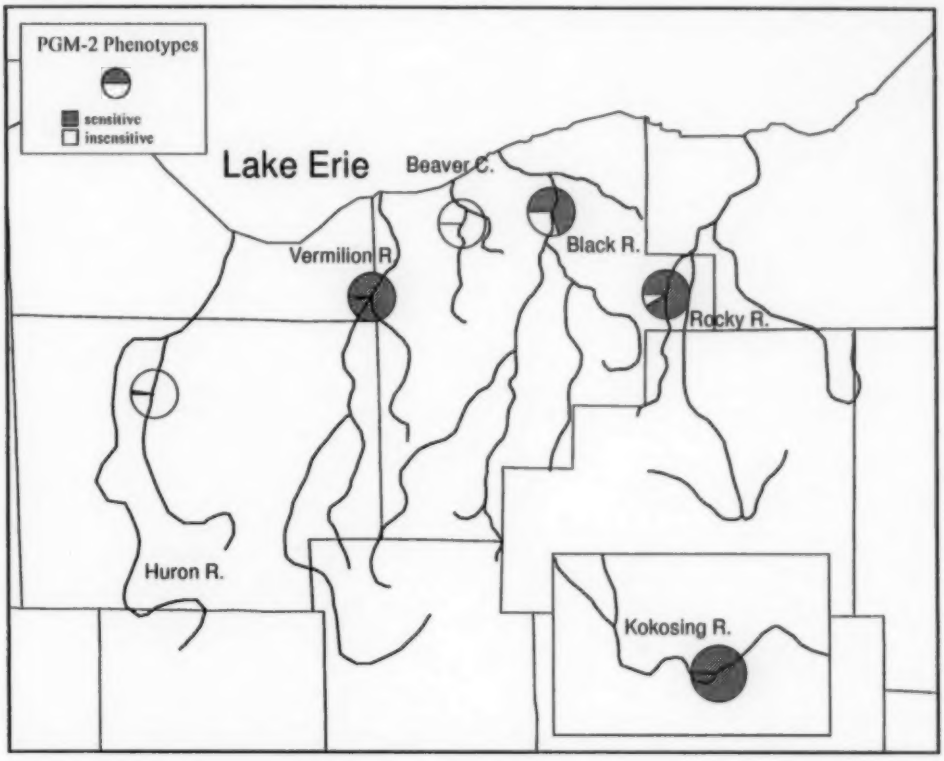

Table 8. Risk of genetic erosion in stream fishes based on ecological characters and pollution tolerance.

Risk	Traits	Example
High	Specialist, pollution intolerant	*Etheostoma caeruleum* and *E. flabellare*
Moderate	Specialist and pollution tolerant or intermediate specialization and pollution intolerant	*Etheostoma nigrum*
Low	Intermediate specialization and pollution tolerant	*Etheostoma blennoides*
	Generalist and pollution moderately (in)tolerant	*Campostoma anomalum*
Very low	Generalist, pollution tolerant	*Rhinichthys atratulus*

Note: Ecological specialization and pollution tolerance data for fish species are from Ohio EPA (1989).

Water quality monitoring stations would only be feasible at a small number of sites. If positioned too far upstream, they might miss important pollution events. If positioned downstream, it may be impossible to determine the origin of pollutants. Electrophoretic sampling would allow a much larger area of a river to be monitored and would allow more precise identification of pollution sources.

Implications for species conservation

We have presented a scenario in which ecology, life history, pollution tolerance, and water quality affect genetic variation. Small population size, a high degree of specialization, low pollution tolerance, and poor water quality are linked to low genetic variation. How do we use this information to aid in conservation and management decisions for a particular species? First, we must not forget about the complex interplay of all of these parameters. Degree of habitat specialization, pollution tolerance, and water quality can affect the absolute and relative population size, leading to changes in genetic structure. Water quality can directly erode variation through directional selection. Consideration of possible additive effects allows us to classify species according to risk of genetic erosion (Table 8). Combining our understanding of how ecology and habitat specialization relate to genetic variation should be of value in designing management strategies, even when direct genetic data are not available.

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Appendix 1. Allele frequencies of all polymorphic loci in *Etheostoma caeruleum*.

	Population												
Locus	H1	H2	H3	H4	K1	K3	K4	R1	V1	V2	V3	V4	B2
ADH-1													
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.958	1.000	1.000	1.000	1.000
GPI-1													
*1	0.021	0.021	0.000	0.000	0.979	1.000	0.979	0.021	0.042	0.000	0.021	0.000	0.000
*2	0.979	0.979	1.000	1.000	0.021	0.000	0.021	0.979	0.958	1.000	0.979	1.000	1.000
LHD-1													
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.021	0.083	0.021	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.938	.0979	0.917	0.979	1.000
MDH-1													
*1	1.000	1.000	1.000	1.000	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000	1.000
*2	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2													
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000
MDH-4													
*1	0.979	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*2	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MEP-1													
*1	0.042	0.125	0.063	0.000	0.000	0.042	0.000	0.042	0.167	0.042	0.104	0.000	0.000
*2	0.958	0.875	0.938	1.000	1.000	0.958	1.000	0.938	0.833	0.958	0.896	1.000	1.000
*3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
MPI-1													
*1	1.000	1.000	1.000	1.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
*2	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
PGDH-1													
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
*2	1.000	1.000	1.000	1.000	0.979	0.979	0.958	1.000	1.000	1.000	0.979	1.000	1.000
*3	0.000	0.000	0.000	0.000	0.021	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*4	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1													
*1	0.000	0.021	0.021	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071
*2	1.000	0.979	0.979	0.979	0.938	0.938	0.896	1.000	1.000	1.000	1.000	1.000	0.929
*3	0.000	0.000	0.000	0.000	0.063	0.063	0.104	0.000	0.000	0.000	0.000	0.000	0.000

Note: Loci were considered polymorphic if more than one allele was found within the species. B, Black River; H, Huron River; K, Kokosing River; R, Rocky River; V, Vermilion River.

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Appendix 2. Allele fequencies of all polymorphic loci in *Etheostoma blennioides*.

	Population															
Locus	B1	B2	B3	H1	H2	H3	H4	K1	K2	K3	K4	R1	V1	V2	V3	V4
GPI-1																
*1	0.083	0.125	0.271	0.021	0.063	0.022	0.021	0.188	0.205	0.188	0.271	0.083	0.063	0.063	0.021	0.104
*2	0.917	0.875	0.729	0.979	0.938	0.978	0.979	0.813	0.795	0.813	0.729	0.917	0.938	0.938	0.979	0.896
IDHP-1																
*1	1.000	1.000	0.938	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.938	1.000
*2	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000
MDH-1																
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
*2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
*3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.958	1.000	1.000	1.000	1.000	1.000
MDH-2																
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.979	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000
*3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3																
*1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.977	1.000	1.000	0.979	1.000	1.000	1.000	1.000
*2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.021	0.000	0.000	0.000	0.000
MDH-4																
*1	0.208	0.167	0.083	0.000	0.000	0.000	0.000	0.083	0.318	0.208	0.292	0.000	0.063	0.000	0.333	0.000
*2	0.063	0.083	0.167	0.000	0.000	0.000	0.000	0.083	0.068	0.125	0.063	0.000	0.000	0.063	0.042	0.021
*3	0.729	0.750	0.750	1.000	1.000	1.000	1.000	0.833	0.614	0.667	0.646	1.000	0.938	0.938	0.625	0.979
MEP-1																
*1	0.021	0.333	0.313	0.042	0.042	0.022	0.021	0.417	0.159	0.021	0.333	0.104	0.042	0.042	0.104	0.104
*2	0.271	0.083	0.125	0.021	0.063	0.022	0.083	0.083	0.227	0.458	0.146	0.021	0.271	0.083	0.083	0.042
*3	0.708	0.583	0.563	0.938	0.896	0.957	0.896	0.500	0.614	0.521	0.521	0.875	0.688	0.875	0.813	0.854
MPI-1																
*1	0.125	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*2	0.833	0.958	0.958	1.000	1.000	1.000	1.000	0.958	1.000	0.917	1.000	1.000	0.979	0.958	0.958	1.000
*3	0.042	0.021	0.042	0.000	0.000	0.000	0.000	0.042	0.000	0.083	0.000	0.000	0.021	0.042	0.042	0.000
PGDH-1																
*1	0.000	0.000	0.000	0.000	0.000	0.043	0.042	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	0.957	0.958	1.000	1.000	1.000	1.000	0.917	1.000	1.000	1.000	1.000
PGM-1																
*1	1.000	1.000	0.979	1.000	1.000	0.957	1.000	0.979	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*2	0.000	0.000	0.021	0.000	0.000	0.043	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Loci were considered polymorphic if more than one allele was found within the species. B, Black River; H, Huron River; K, Kokosing River; R, Rocky River; V, Vermilion River.

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Appendix 3. Allele frequencies of all polymorphic loci in *Campostoma anomalum*.

	Population															
Locus	B2	B3	B4	H1	H2	H3	H4	K1	K3	K4	R1	V1	V2	V3	V4	BC
EST-1																
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.045	0.261	0.313	0.188	0.250	0.229	0.159	0.000
*2	0.563	0.000	1.000	0.000	0.000	0.000	0.000	0.152	0.136	0.304	0.208	0.167	0.167	0.083	0.114	0.000
*3	0.313	0.611	0.000	1.000	1.000	1.000	1.000	0.543	0.545	0.435	0.479	0.313	0.229	0.354	0.477	1.000
*4	0.125	0.389	0.000	0.000	0.000	0.000	0.000	0.283	0.273	0.000	0.000	0.333	0.354	0.333	0.250	0.000
EST-2																
*1	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*2	1.000	0.857	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1																
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000
GPI-2																
*1	0.000	0.056	0.000	0.104	0.063	0.083	0.083	0.063	0.045	0.042	0.208	0.063	0.042	0.104	0.125	0.063
*2	1.000	0.944	1.000	0.896	0.938	0.917	0.917	0.938	0.955	0.958	0.792	0.938	0.958	0.896	0.875	0.938
MEP-1																
*1	0.000	0.000	0.000	0.000	0.042	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*2	0.625	0.167	0.531	0.917	0.958	0.958	1.000	1.000	0.977	0.938	0.792	0.750	0.833	0.792	0.739	0.125
*3	0.125	0.389	0.313	0.083	0.000	0.000	0.000	0.000	0.023	0.063	0.188	0.146	0.063	0.208	0.152	0.000
*4	0.250	0.444	0.156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.063	0.104	0.000	0.109	0.875
PGDH-2																
*1	0.104	0.111	0.167	0.000	0.000	0.021	0.000	0.146	0.068	0.333	0.146	0.083	0.104	0.167	0.146	0.125
*2	0.458	0.500	0.271	0.000	0.000	0.000	0.000	0.854	0.932	0.667	0.708	0.792	0.792	0.563	0.708	0.875
*3	0.458	0.389	0.563	1.000	1.000	0.979	1.000	0.000	0.000	0.000	0.146	0.125	0.104	0.271	0.146	0.000
PGDH-3																
*1	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.104	0.045	0.104	0.021	0.104	0.021	0.042	0.021	0.000
*2	1.000	1.000	0.958	1.000	1.000	1.000	1.000	0.896	0.955	0.896	0.979	0.896	0.979	0.958	0.979	1.000
PGM-1																
*1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-2																
*1	0.438	0.556	0.438	0.021	0.021	0.000	0.000	0.771	0.932	0.833	0.708	0.875	0.771	0.833	0.667	0.000
*2	0.563	0.389	0.563	0.979	0.958	1.000	1.000	0.229	0.068	0.167	0.292	0.125	0.229	0.167	0.333	1.000
*3	0.000	0.056	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Loci were considered polymorphic if more than one allele was found within the species. B, Black River; H, Huron River; K, Kokosing River; R, Rocky River; V, Vermilion River.

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Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies

Arnulf Soleng and Tor A. Bakke

Abstract: The salinity tolerance of the freshwater monogenean *Gyrodactylus salaris*, infecting Atlantic salmon (*Salmo salar*) parr, was studied experimentally. Following direct transfer of infected fish from fresh water to 5.0‰ salinity, parasite population growth increased at the same rate as in fresh water and was positively correlated with temperature (1.4, 6.0, and 12.0°C). In 7.5‰ salinity the populations declined and became extinct after a maximum of 56 days, without any significant difference between 6.0 and 12.0°C. However, some infrapopulations demonstrated short periods of growth. At higher salinities (10.0, 15.0, 20.0, and 33.0‰) the survival time decreased, and there was a negative correlation between survival time and temperature (1.4, 6.0, and 12.0°C). When transferred directly to sea water (33.0‰) the parasites became opaque and ceased moving after a few minutes. There was no difference in parasite survival time between direct and gradual transfer from fresh water to 7.5 and 10.0‰, except for one infrapopulation which demonstrated population growth from day 22 after some fluctuations following gradual transfer to 7.5‰. The present findings support the hypothesis of brackish water dispersal of *G. salaris* with infected fish migrating between rivers in fjord systems.

Résumé : La tolérance à la salinité du monogénien d'eau douce *Gyrodactylus salaris*, qui infecte les tacons de saumon atlantique (*Salmo salar*), a été étudiée expérimentalement. Après transfert direct des poissons infectés de l'eau douce à une eau présentant une salinité de 5,0 ‰, la croissance de la population de parasites a augmenté à la même vitesse que dans l'eau douce et cette dernière présentait une corrélation positive avec la température (1,4, 6,0 et 12,0°C). À une salinité de 7,5 ‰, les populations ont décliné jusqu'à extinction complète après un maximum de 56 jours, sans qu'il y ait de différence statistiquement significative entre 6,0 et 12,0°C. Toutefois, certaines sous-populations ont présenté de courtes périodes de croissance. À des salinités plus élevées (10,0, 15,0, 20,0 et 33,0‰), la durée de survie a diminué et on a constaté une corrélation négative entre la durée de survie et la température (1,4, 6,0 et 12,0°C). Lorsqu'ils ont été transférés directement dans l'eau de mer (33,0‰), les parasites sont devenus opaques et se sont immobilisés après quelques minutes. On n'a pas observé de différence dans la durée de survie des parasites entre le transfert direct et le transfert graduel de l'eau douce vers une eau de salinité de 7,5 et 10,0‰, sauf dans le cas d'une sous-population qui a montré une croissance de population à partir du jour 22 après certaines fluctuations suivant le transfert graduel à 7,5‰. Les présentes constatations viennent appuyer l'hypothèse de la dispersion de *G. salaris* dans l'eau saumâtre par les poissons infectés migrant entre les rivières dans les systèmes de fjords.
[Traduit par la Rédaction]

Introduction

Monogeneans of the genus *Gyrodactylus* are widespread ectoparasites of both freshwater and marine fishes. Their influence in fish farms can be extensive (Malmberg 1957, 1989*a*, 1989*b*; Bauer 1958, 1988; Cone et al. 1983; Musselius 1988; Solomatova and Luzin 1988). There are few reports of overt disease in natural waters caused by gyrodactylids, but in Norway the freshwater species *Gyrodactylus salaris* has caused epidemics that have devastated stocks of Atlantic salmon (*Salmo salar*) in many rivers (see Heggberget and Johnsen 1982; Johnsen and Jensen 1986, 1988, 1991, 1992).

In Norway, *G. salaris* was first observed in natural populations of salmon parr in the River Lakselva in northern Norway in 1975 (Johnsen 1978). By the end of 1994 the parasite had been recorded from 38 rivers and 38 fish farms throughout the country (Soleng and Bakke 1995).

Generally, most parasites are disseminated and introduced into new localities by movements of infected hosts (Kennedy 1976), naturally or by human activity (Kennedy 1993). Because the ease of colonization and establishment of a new locality is inversely related to the life-cycle complexity (Kennedy 1993), monogenic parasites like *G. salaris* have a relatively great potential for colonization. Malmberg (1993) suggested that *G. salaris* once spread from central Asia to the Baltic area of Europe. Besides Norway, *G. salaris* is also known from Sweden, Denmark, Finland (Malmberg and Malmberg 1993), Russia, Ukraine, Georgia, Crimea (Malmberg 1993), Germany (Lux 1990), Spain (Malmberg 1993), France, and Portugal (T.A. Mo, National Veterinary Institute, Oslo, Norway, personal communication). Johnsen and Jensen (1986) found the distribution of *G. salaris* in Norway to be related to stocking of fish from infected hatcheries and suggested that *G. salaris* is most probably a recent introduction to Norway. Later, Halvorsen and Hartvigsen (1989) disputed this theory even though they found the association between the

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A. Soleng[1] **and T.A. Bakke.** Zoological Museum, University of Oslo, Sars gate 1, N-0562 Oslo, Norway.

[1] Author to whom all correspondence should be addressed.
e-mail: arnulf.soleng@toyen.uio.no

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Table 1. Experimental protocol for the gradual transfer of *Gyrodactylus salaris* infected Atlantic salmon (*Salmo salar*) parr from fresh water to 7.5 and 10.0‰ salinity at 12.0°C.

	Salinity (‰)					
Experiment	5.0	6.2	7.5	8.2	9.1	10.0
FW to 7.5‰	16 days	—	Test salinity			
FW to 10.0‰ (No. 1)	4 days	—	2 days	—	—	Test salinity
FW to 10.0‰ (No. 2)	1 day	1 day	1 day	1 h	1 h	Test salinity

Note: The fish were directly transferred from fresh water to 5.0‰ salinity. Test salinity, the salinity at which the infrapopulation survival was assessed; FW, fresh water; —, fish were not exposed to this salinity.

distribution of *G. salaris* and stocking of fish to be significant. The possibility of natural dispersal by migration of infected fish through fjord systems to neighbouring rivers has also been noted (Anonymous 1982; Johnsen and Jensen 1986; Lund and Heggberget 1992).

Brackish water, with a salinity between 0.5 and 30‰, is physiologically an important barrier to distribution and range extension for both freshwater and marine species (Schmidt-Nielsen 1989). Malmberg (1970) found gyrodactylids in sea, brackish, and fresh water and indicated that the level of salinity tolerance varies among species. Several freshwater species tolerate an increase in salinity to at least 6‰, and many seawater species tolerate a decrease from 35 to at least 6‰ (Malmberg 1957, 1970). Details concerning the salinity tolerance of *G. salaris* is limited (Anonymous 1981; Mo 1987; Malmberg 1988, 1989*b*).

Environmental temperature is a further important variable, both for the host and the parasite. Generally, the survival time of monogeneans increases with decreasing temperature (Lester and Adams 1974; Chubb 1977; Scott and Nokes 1984; Jansen and Bakke 1991). The ability of animals to osmoregulate is influenced by temperature, and may cease below and above certain critical temperatures (Tait 1980). However, the influence of temperature on salinity tolerance in *G. salaris* has not been studied.

The aim of this study was, therefore, to experimentally study the salinity tolerance of *G. salaris* on Atlantic salmon parr, and its relationship to ambient temperature. This was done by measuring population growth and survival under different salinity and temperature regimes, after both direct and gradual transfer from fresh water.

Materials and methods

Hatchery-reared Atlantic salmon parr (age 1+; mean length 7.4 cm (range 4.4–10.5 cm); mean weight 3.6 g (range 0.6–8.8 g)), of the River Lierelva stock, Buskerud County in southeast Norway, were used in the experiments. These fish had not previously been exposed to any *Gyrodactylus* infection and had been routinely disinfected against ectoparasites and fungus in the hatchery.

The *G. salaris* used in the experiments originated from naturally infected salmon parr electrofished in the River Lierelva. These fish were kept in the laboratory at 12–13°C in fish tanks ($1 \times 1 \times 0.3$ m water level) with a continuous flow of active-charcoal filtered and dechlorinated laboratory fresh water (1–2 $L \cdot min^{-1}$). Aquarium power filters (Fluval 403 without filter medium; 1200 $L \cdot h^{-1}$) circulated the water and created uniform water temperatures in the tanks. Any dead fish were replaced with previously uninfected hatchery fish or newly caught, naturally infected fish. The infected fish were marked by adipose fin clipping and used to infect hatchery fish by placing infected and uninfected fish together in grey plastic boxes ($0.38 \times 0.27 \times 0.12$ m water level) for different times (1–3 days) depending on the infection level needed.

The fish were acclimated to the experimental water temperatures of 1.4°C (range 1.1–1.7°C), 6.0°C (range 5.7–6.3°C), and 12.0°C (range 11.6–12.4°C) for a minimum of 1 week prior to each experiment. The temperature was continuously measured with a thermometer with maximum and minimum memory.

Natural sea water, with a salinity of approximately 33.0‰ (YSI model 33 salinometer), was taken from a depth of 50 m at Drøbak (Biological Station, University of Oslo) in the Oslofjord, filtered (Schleicher & Schull paper filter, Black ribbon 589, pore size 7–12 m), and diluted with laboratory water at pH ca. 6.5 (Orion model 230A pH meter) to the required salinities (5.0‰, pH 7.3; 7.5‰, pH 7.4; 10.0‰, pH 7.6; 15.0, 20.0, and 33.0‰, pH 7.9; all pH measurements were performed at 12.0°C).

Four grey plastic boxes were placed floating in each fish tank, except at 1.4°C where the boxes were placed in a refrigerated room. Each box was divided into three equal compartments. One fish was individually isolated in each compartment, so 12 infected fish were tested concurrently at each salinity and temperature, except in sea water (33.0‰) where the fins from 6 fish were used. A transparent lid was placed over the boxes to reduce evaporation and subsequent changes in the salinity. About 95% of the water in each box was changed every third day, and all the boxes were aerated. To dim the constant illumination a semitransparent lid was placed over the fish tanks. During the experiments the fish were not fed.

Growth and survival of infrapopulations of *G. salaris* was assessed by counting the *G. salaris* specimens on the external skin and fins of anaesthetized fish (cf. Bakke et al. 1991), except at 33.0‰ where no anaesthesia was used. Anaesthesia may be stressful to the parasites. However, Harris et al. (1994) stressed that the mortality and fecundity schedules of *G. salaris* derived by Jansen and Bakke (1991) are close to those occurring in natural infections, despite the complications of frequent anaesthesia. Both the anaesthesia and the laboratory water used had the same temperature and salinity as in the respective experiments and were changed between each examined fish.

Experiment 1: direct transfer from fresh water to salt water

The growth and survival of infrapopulations of *G. salaris* was measured after direct transfer of infected fish (or fins) from fresh (0.0‰) to brackish (5.0, 7.5, 10.0, 15.0, and 20.0‰) and sea water (33.0‰). The experiments were performed at 1.4, 6.0, and 12.0°C, except at 7.5‰ where the lowest temperature was omitted because of the long time scale of the experiment. The survival time of an infrapopulation was defined as the time from the beginning of the experiment until death or loss of all parasites from the fish.

Experiment 2: gradual transfer from fresh water to salt water

The growth and survival of infrapopulations of *G. salaris* was measured after gradual transfer of infected fish from fresh water (0.0‰) to 7.5 and 10.0‰ salinity at 12.0°C (see Table 1). The survival time of an infrapopulation was defined as the time from the beginning of exposure to 7.5 or 10.0‰ until death or loss of all parasites from the fish. All results were compared with the direct transfer to 7.5 and 10.0‰ at 12.0°C.

The statistical tests used are in accordance with Zar (1984), and the level of significance was 5% ($\alpha = 0.05$). The parasitological terms infrapopulation, intensity, mean intensity, and prevalence are in accordance with Margolis et al. (1982). In experiments where populations declined and were eliminated, the data were standardized as percentages of initial infrapopulation size. To allow these data to be plotted on a log scale, 1 was added to each value. Thus, the term

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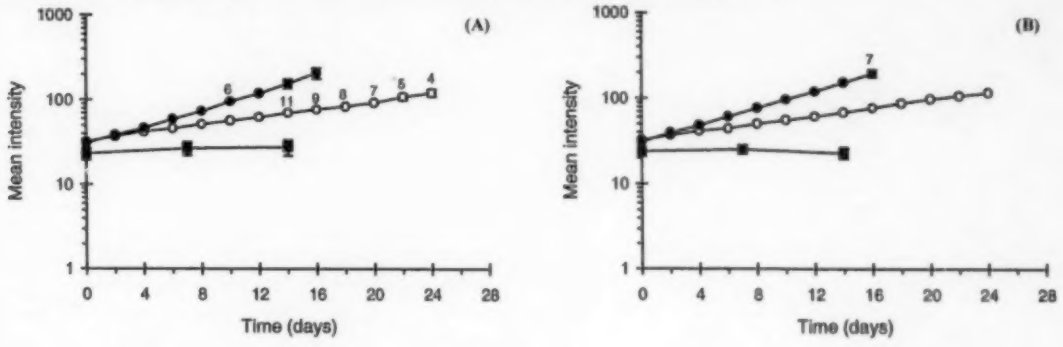
Fig. 1. The course of infection of *Gyrodactylus salaris* on individually isolated Atlantic salmon (*Salmo salar*) parr. (A) Fresh water (0.0‰ salinity). (B) 5.0‰ salinity after direct transfer from fresh water. (■) 1.4°C, $n = 12$; (○) 6.0°C, $n = 12$; (●) 12.0°C, $n = 12$. Changes in n are indicated by numbers close to data points. Error bars are SEs of the arithmetic mean. Note $\log_{10}$ scale.



population index$_t$, which is plotted on the Y axes in Figs. 3–5, is defined as follows:

$$\text{Population index}_t = \sum^{n_t} \frac{P_{it} \times \frac{100}{p_{i0}}}{n_t} + 1$$

where P_{it} is infrapopulation size at time t, P_{i0} is infrapopulation size at time 0 (start of experiment), and n_t is number of fish at time t.

Results

Experiment 1: direct transfer from fresh water to salt water

There was population increase of *G. salaris* on all fish at both 1.4, 6.0, and 12.0°C during the experiments in fresh water (Fig. 1A). The mean intensity of infection increased from 23.0 to 27.5 during 14 days at 1.4°C and from 31.8 to 121.8 during 24 days at 6.0°C. At 12.0°C a mean intensity of 30.7 increased to 206.2 during the 16 days of the experiment. The rate of population growth was, therefore, positively related to temperature.

During the experiments in 5.0‰ salinity, there was also population increase on all fish at 6.0 and 12.0°C (Fig. 1B). The mean intensity increased from 32.3 to 119.3 over 24 days at 6.0°C and from 30.6 to 198.7 after 16 days at 12.0°C. At 1.4°C the mean intensity increased during the first week from 23.8 to 25.1 but was then reduced to 24.8 during the second week (Fig. 1B). At 5.0‰ salinity the rate of population growth was positively related to temperature. No main difference in population growth rate was observed between fresh water and 5.0‰ salinity.

Overall, there was a decrease in the number of *G. salaris* at both 6.0 and 12.0°C in 7.5‰ salinity, despite short periods of population growth (Figs. 2A and 2B). The rate of decrease was almost constant (Figs. 3A and 3B), and large differences between infrapopulation survival times were observed at the same temperature (Table 2). There was no significant difference in survival time between 6.0 and 12.0°C (Mann–Whitney U test, $0.20 > p > 0.10$).

Populations decreased in salinities of 10.0, 15.0, and 20.0‰ at all temperatures tested (1.4, 6.0, and 12.0°C) (Figs. 4A–4F), and the survival time was negatively correlated with temperature (Table 2, Figs. 4A–4F).

During the first few minutes after direct transfer of infected fins to sea water (33.0‰) the parasites showed prominent "searching or reaching activity" (see Harris 1982), which increased with temperature. During this activity the body of the parasite is extended considerably, and the behaviour is believed to have an exploratory and sensory function (Harris 1982). The parasites then turned opaque after several minutes (negatively related to temperature), and all movement ceased (Table 2). The parasites were, however, not necessarily dead (cf. Soleng et al. 1997).

Experiment 2: gradual transfer from fresh water to salt water

No significant difference in infrapopulation survival time (Table 2) between gradual and direct transfer to 7.5‰ salinity was observed (Mann–Whitney U test, $p = 0.10$), although the population decrease was more rapid after gradual transfer during the first days (Figs. 3A and 3B). However, one infrapopulation showed periods of both increase and decrease during the first 22 days, followed by steady population growth to day 40 when the fish suddenly died (Fig. 2C).

No significant difference in infrapopulation survival time (Table 2) between gradual and direct transfer to 10.0‰ salinity was observed (Kruskal–Wallis, $0.10 > p > 0.05$) (Figs. 5A and 5B).

Infrapopulation size and survival time

There were no significant correlations between the sizes of infrapopulations at the beginning of the experiments and their survival times, except in the direct transfer to 15.0‰ salinity at 1.4°C where the survival times increased with increasing infrapopulation size (Table 2).

Discussion

The population growth of *G. salaris*, positively correlated with temperature, was similar in fresh water and 5.0‰ salinity. This is in accordance with results from other flatworms, e.g., the survival time at different salinities of cercariae of the genus *Schistosoma* was similar up to 5.25‰ (Donnelly et al. 1984), but the authors generally recorded a prolonged survival time in salinities of 1.75, 3.5, and 5.25‰ compared with fresh water. The maximum survival time of *Schistosoma haematobium* and

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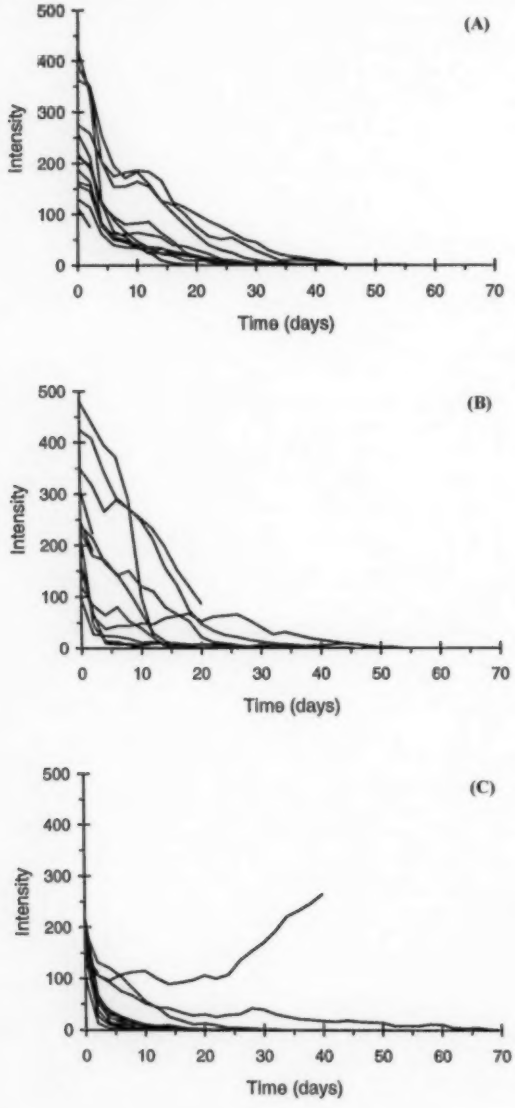
Fig. 2. The course of infection of *Gyrodactylus salaris* on 12 individually isolated Atlantic salmon (*Salmo salar*) parr in 7.5‰ salinity. (A) Individual trajectories after direct transfer from fresh water at 6.0°C. (B) Individual trajectories after direct transfer from fresh water at 12.0°C. (C) Individual trajectories after gradual transfer (direct from fresh water to 5.0‰, after 16 days transferred to 7.5‰) at 12.0°C.



Schistosoma mansoni was found to occur in a salinity of 3.5‰. *Schistosoma mattheei* showed maximum survival time in 1.75‰, but there were also prolonged survival times of some cercariae in salinities of 3.5 and 5.25‰. The longer survival time of the nonfeeding cercariae in 3.5 and 1.75‰ salinity, compared with fresh water, is suggested to be due to lower osmoregulation energy use because of a lower osmotic gradient between the cercariae and the external environment (Donnelly et al. 1984). For *G. salaris*, the present results imply that the isoosmotic point is between 0.0 and 7.5‰ salinity. Theoretically, the use of energy to sustain the osmotic and ionic balance are lowest at this point, which would suggest a higher potential for population growth and perhaps longer survival of individual parasites than in fresh water.

The present recorded survival and reproduction of *G. salaris* in 5.0‰ salinity are in accordance with Malmberg (1957, 1970), who found that many freshwater species of *Gyrodactylus* tolerate an increase in salinity up to at least 6‰. In fish farms in the Baltic Sea, *G. salaris* was found to survive and possibly reproduce in salinities of approximately 5‰ on rainbow trout, *Oncorhynchus mykiss* (Malmberg 1988, 1989*b*). In addition, Mo (1987) found that *G. salaris* survived at least 48 h in 6‰ salinity at 10°C on Atlantic salmon parr in laboratory experiments.

A marked change in the salinity tolerance of *G. salaris* occurred between 5.0 and 7.5‰ salinity. In 5.0‰ the population growth increased, while in 7.5‰ the infrapopulations were lost. In 7.5‰ the mean survival time was 38.0 and 33.1 days at 6.0 and 12.0°C, respectively. This is in accordance with Donnelly et al. (1984) who found a marked reduction in the survival time for *Schistosoma* cercariae in 7.0‰ salinity compared with 5.25‰. In our experiments some infrapopulations of *G. salaris* increased in size for short periods in 7.5‰, both at 6.0 and 12.0°C, indicating population growth. This, however, could be the result of reattachment of shed parasites and not parasite reproduction. Nevertheless, Jansen and Bakke (1991) found that the maximum survival time for *G. salaris* individuals on salmon parr, in fresh water, was 59 days at 6.6°C and 25 days at 13°C. This implies that parasite reproduction must have occurred if the duration of a *G. salaris* infection continues longer than this. Thus, the maximum recorded survival times in 7.5‰ salinity of 56 days (6.0°C) and 54 days (12.0°C) also indicate that reproduction occurred, especially at 12.0°C.

There was a marked decrease in survival time with increasing salinity at 10.0, 15.0, 20.0, and 33.0‰. No reproduction of *G. salaris* was observed at these salinities. However, the survival time was negatively correlated with temperature. In contrast to previous findings (Anonymous 1981), where *G. salaris* infections were eliminated in a salinity of 15‰ after 12 h (temperature not indicated), the present results demonstrate that at 12.0°C the mean survival time for infrapopulations is 17.3 h (maximum 24 h). This is also longer than observed for *Schistosoma* cercariae (Donnelly et al. 1984). In our seawater experiments (33.0‰), *G. salaris* individuals became opaque and immobile after a mean time of 9 min (12.0°C), 17 min (6.0°C), and 18 min (1.4°C). These results also contrast with previous observations (Anonymous 1981) where *G. salaris* died after 6–8 min in salinities over 30‰ (temperature not indicated). Our seawater experiments were carried out with infected fins from deliberately killed hosts, allowing continuous observation of the parasites. Nevertheless, the survival time of *G. salaris* on dead hosts in fresh water (Mo 1987) is far longer than the present observations in sea water.

It was difficult to judge whether or not *G. salaris* were dead during the exposure to sea water, or if the parasites only became inactive due to osmotic stress. Soleng et al. (1997) examined this by exposing infected salmon parr to brackish and sea water for different time periods before returning them to fresh water. This demonstrated that an exposure to sea water

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Fig. 3. The course of infection of *Gyrodactylus salaris* on individually isolated Atlantic salmon (*Salmo salar*) parr in 7.5‰ salinity. (A) Summary of course of infection. (B) Prevalence. (○) Direct transfer from fresh water at 6.0°C, $n = 12$. (●) Direct transfer from fresh water at 12.0°C, $n = 12$. (■) Gradual transfer (direct from fresh water to 5.0‰, after 16 days transferred to 7.5‰), $n = 11$; one infrapopulation demonstrating population growth from day 22 was not included. Changes in n are indicated by numbers close to data points in A. Error bars are SEs of the arithmetic mean. Note $\log_{10}$ scale for A. See text for definition of population index$_t$.

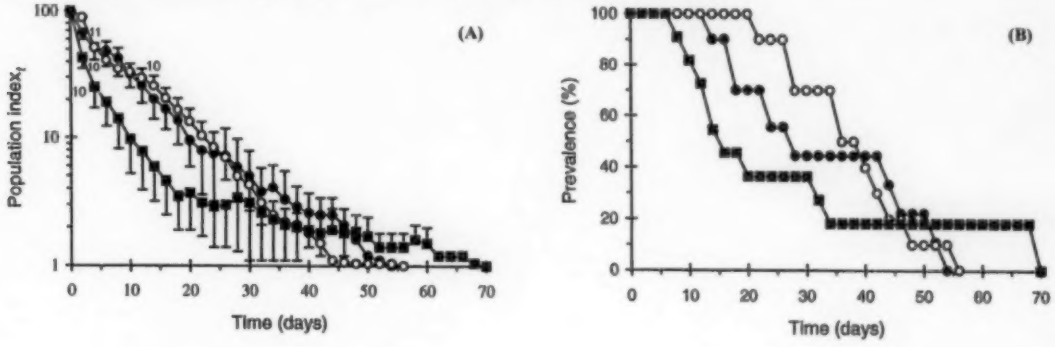

Table 2. Mean survival time and intensity (at the start of the experiment) for infrapopulations of *Gyrodactylus salaris* on Atlantic salmon (*Salmo salar*) parr in different salinities at a range of temperatures.

Transfer	Salinity (‰)	Temperature (°C)	Survival time	Intensity	n	r_s	p
Direct	0.0	1.4	Population growth	23.0±3.7 (12–55)	12	—[a]	—[a]
	0.0	6.0	Population growth	31.8±0.8 (28–35)	4	—[a]	—[a]
	0.0	12.0	Population growth	30.7±0.5 (28–33)	6	—[a]	—[a]
	5.0	1.4	Population growth	23.8±3.1 (12–47)	12	—[a]	—[a]
	5.0	6.0	Population growth	32.3±0.6 (28–36)	12	—[a]	—[a]
	5.0	12.0	Population growth	30.6±1.0 (26–36)	7	—[a]	—[a]
	7.5	6.0	38.0±3.2 days (22–56)	240.6±29.9 (112–422)	10	0.317	>0.20
	7.5	12.0	33.1±5.3 days (14–54)	255.2±34.3 (95–480)	9	–0.301	>0.20
	10.0	1.4	229.1±3.8 h (216–240)	255.0±18.9 (108–306)	11	0.231	>0.50
	10.0	6.0	117.0±2.2 h (108–132)	318.3±35.6 (110–475)	12	0.071	>0.50
	10.0	12.0	64.8±1.7 h (60–72)	141.3±19.7 (66–249)	10	0.099	>0.50
	15.0	1.4	64.0±2.8 h (54–78)	203.3±18.0 (102–330)	9	0.807	<0.02
	15.0	6.0	32.4±2.7 h (24–54)	208.9±18.0 (124–312)	10	0.566	>0.05
	15.0	12.0	17.3±0.8 h (15–24)	397.1±22.6 (313–540)	12	–0.011	>0.50
	20.0	1.4	36.5±1.0 h (30–42)	160.8±9.2 (123–210)	10	–0.087	>0.50
	20.0	6.0	16.3±0.7 h (12–21)	165.5±12.4 (108–239)	12	0.408	>0.10
	20.0	12.0	10.5±0.5 h (9–12)	433.2±37.3 (241–715)	12	–0.386	>0.20
	33.0	1.4	18±0.8 min (15–20)[b]	—[a] (80–100)	6	—[a]	—[a]
	33.0	6.0	17±0.7 min (15–19)[b]	—[a] (80–100)	6	—[a]	—[a]
	33.0	12.0	9±0.3 min (8–10)[b]	—[a] (80–100)	6	—[a]	—[a]
Gradual	7.5	12.0	23.0±5.9 days (8–70)[c]	160.4±10.6 (99–217)	11	0.377	>0.20
No. 1	10.0	12.0	58.2±1.8 h (48–66)	64.4±7.5 (38–123)	10	0.075	>0.50
No. 2	10.0	12.0	59.5±1.9 h (48–66)	126.2±19.9 (40–235)	11	0.467	>0.10

Note: Values are mean ± SE, with range given in parentheses. Spearman rank correlation (r_s) was used to analyse the relationship between infrapopulation size at the beginning of the experiments and survival time. n, number of fish at the end of the experiment ($n < 12$ indicates fish death, except at 33.0‰ where $n = 6$ at the start).

[a]Not examined.

[b]Mean time until the parasites became opaque and immobile but not necessarily dead (see text).

[c]One infrapopulation demonstrated continuous population growth after 22 days until death of host (see text).

for 30 min did not kill all parasites. Some survived and even reproduced in fresh water afterwards. Isakov and Shul'man (1956) also observed that *Gyrodactylus arcuatus*, *G. bychowskii*, and *G. rarus* on threespine sticklebacks, *Gasterosteus aculeatus*, and ninespine sticklebacks, *Pungitius pungitius*, became opaque and torpid when transferred directly from fresh to sea water. However, unlike *G. salaris*, all of these euryhaline parasites resumed normal colour and mobility in sea water after some minutes.

Lester and Adams (1974) recorded *Gyrodactylus alexanderi* on threespine sticklebacks in both sea and fresh water. Direct transfer of the infected fish from 30‰ sea water to fresh water (and vice versa) killed the parasites in 30 min. If the transfer was gradual (1 day in 15‰, then 1 day in 8‰ salinity),

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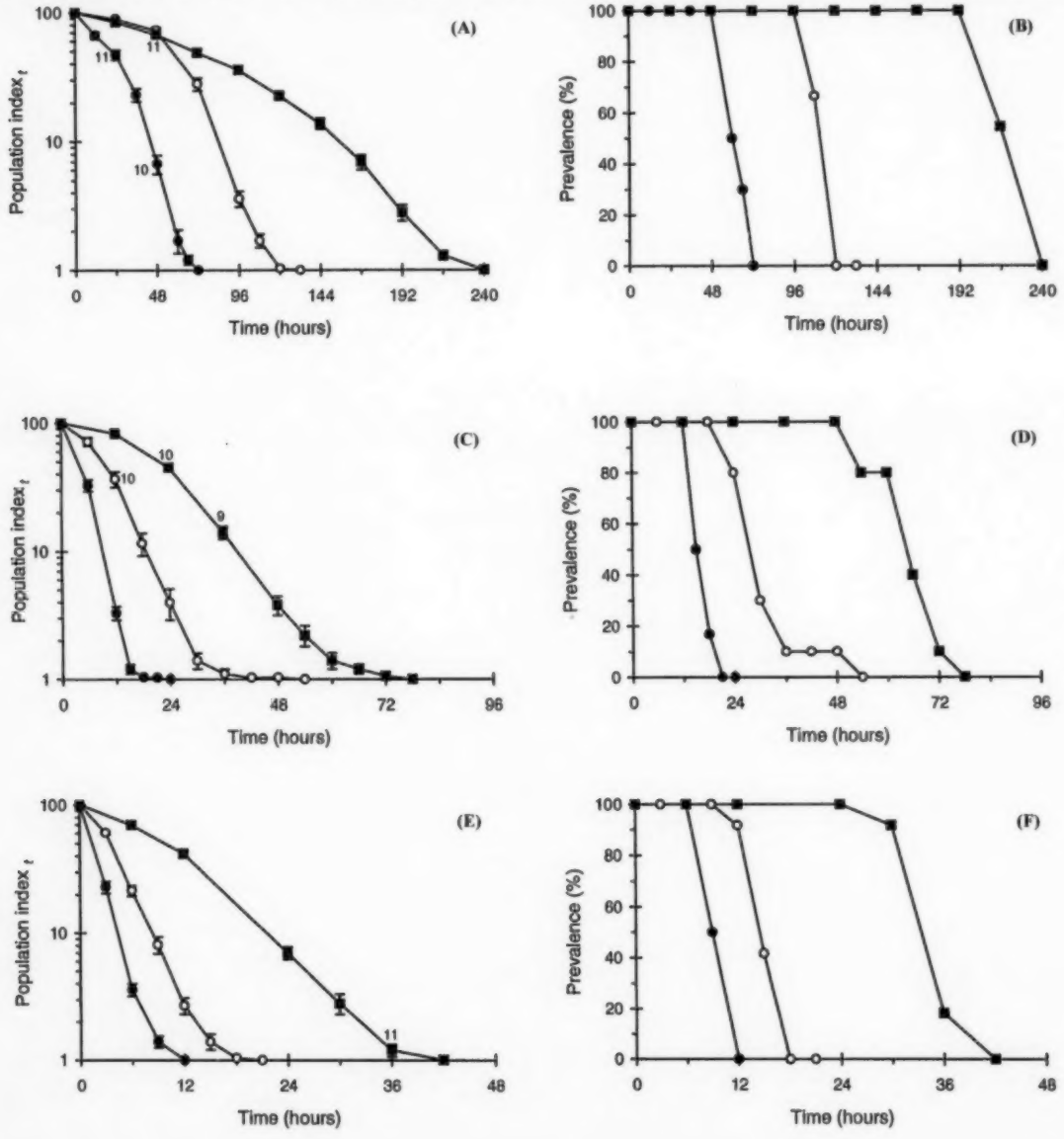
Fig. 4. The course of infection of *Gyrodactylus salaris* on individually isolated Atlantic salmon (*Salmo salar*) parr after direct transfer from fresh water to different salinities. (A) Summary of the course of infection at 10.0‰ salinity. (B) Prevalence at 10.0‰ salinity. (C) Summary of the course of infection at 15.0‰ salinity. (D) Prevalence at 15.0‰ salinity. (E) Summary of the course of infection at 20.0‰ salinity. (F) Prevalence at 20.0‰ salinity. (■) 1.4°C, $n = 12$; (○) 6.0°C, $n = 12$; (●) 12.0°C, $n = 12$. Changes in n are indicated by numbers close to data points in A, C, and E. Error bars are SEs of the arithmetic mean. Note $\log_{10}$ scale for A, C, and E. See text for definition of population index$_t$.



some marine parasites (13.5%) survived at least 7 days in fresh water, and 0.9% of the freshwater parasites survived gradual transfer from fresh to sea water for up to 5 days. In the present study, however, no significant difference was found between direct and gradual transfer, except one fish gradually transferred to 7.5‰ (Fig. 2C).

The variation in survival time between the different infrapopulations was large, both after direct and gradual transfer to 7.5‰ salinity. Also, one of the 11 infrapopulations gradually transferred to 7.5‰ demonstrated population growth after some preliminary fluctuations. This may reflect genetic variations in salinity tolerance. *Gyrodactylus salaris* is reported to be morphologically variable (Malmberg 1987; Mo 1991), and the host specificity is exceptionally broad (Bakke et al. 1992). In addition, there is variation in the population dynamics from river to river (Johnsen and Jensen 1991; Mo 1992; Jansen and Bakke 1993), suggesting the existence of strain differences across the range of the parasite. Furthermore, *G. salaris*

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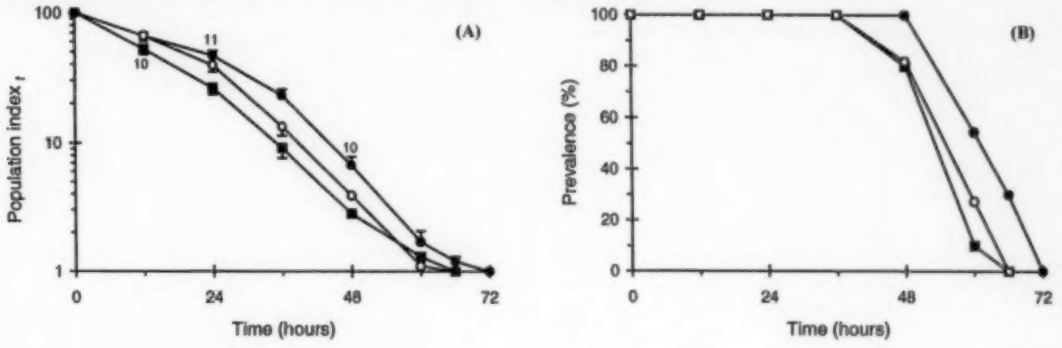
Fig. 5. The course of infection of *Gyrodactylus salaris* on individually isolated Atlantic salmon (*Salmo salar*) parr after direct and gradual transfer to 10.0‰ salinity at 12.0°C. (A) Summary of course of infection at 10.0‰ salinity. (B) Prevalence at 10.0‰ salinity. (■) Gradual transfer No. 2 (direct from fresh water to 5.0‰, 1 day in 5.0‰, 1 day in 6.2‰, 1 day in 7.5‰, 1 h in 8.2‰, 1 h in 9.1‰ before transfer to 10.0‰), $n = 11$; (○) gradual transfer No. 1 (direct from fresh water to 5.0‰, 4 days in 5.0‰, 2 days in 7.5‰ before transfer to 10.0‰), $n = 10$; (●) direct transfer from fresh water to 10.0‰, $n = 12$. Changes in n are indicated by numbers close to data points in A. Error bars are SEs of the arithmetic mean. Note $\log_{10}$ scale for A. See text for definition of population index$_f$.



appears to regularly reproduce sexually, although at low temperatures the populations may contain more newborn asexually derived parasites (Harris et al. 1994). Lynch and Gabriel (1983) have shown that when cyclic parthenogens undergo occasional bouts of sexual reproduction considerable genetic variation may be generated. It would, therefore, be of interest to study the salinity tolerance of *G. salaris* strains from other infected rivers.

Hahnenkamp and Fyhn (1985) reported that salmon lice, *Lepeophtheirus salmonis*, attached to salmon survived hypoosmotic stress longer than free- swimming parasites. They suggested that the death of free-swimming parasites in fresh water was due to an inability to compensate for the loss of body salts and that attached parasites may replace these salts by feeding on the host mucus and body fluids or tissues. The authors also suggest that lice might reduce their rate of salt loss by embedding themselves in the mucus layer of the salmon, thereby increasing the diffusion barrier to the water. Whether or not there is a difference in survival time between attached and free *G. salaris* during hyperosmotic stress is unknown.

The rate of parasite population growth increased with temperature, both in fresh water and 5.0‰ salinity. This is in accordance with Jansen and Bakke (1991), who found temperature important for survival and generation time, number of offspring, and population growth rate of *G. salaris* in fresh water. The survival time for individual parasites was negatively correlated with temperature. The mean number of offspring was optimal between 6.6 and 13.0°C, but the population growth was highest at the highest temperature tested (19.0°C), as the generation time was highly temperature dependent. In the present study the population growth rate in fresh water and 5.0‰ salinity at 6.0 and 12.0°C was similar to what Jansen and Bakke (1991) found for *G. salaris* on individually isolated fish in fresh water at 6.6 and 13.0°C, respectively.

In 7.5‰ salinity, however, no significant difference in survival time between 6.0 and 12.0°C was found. This is in contrast to our results at higher salinities, where an increased temperature reduced the infrapopulation survival time. This discrepancy may be explained by reproduction of *G. salaris* in 7.5‰ salinity. A higher reproductive rate at 12.0°C compared with 6.0°C, as found in fresh water, could compensate for the increased death rate caused by the higher temperature.

There was a clear increase in infrapopulation survival time of *G. salaris* with decreasing temperatures in salinities of 10.0‰ and higher. These results are in accordance with Möller (1978), who also found an increased salinity tolerance of some ectoparasites with decreasing temperature, and the results of Charmantier et al. (1991) on the salinity tolerance of a nemertean worm. Osmotic pressure increases proportionally with temperature (Schmidt-Nielsen 1989), implying that a hypoosmotic animal in sea water will lose water faster at higher temperatures.

Only a few dead *G. salaris* were observed attached to the fish during the salinity tolerance experiments, except on the loose fins in sea water. This may indicate dislodgement of parasites when dying or active abandonment of the host when environmental conditions turn unfavourable. The opisthaptor of *G. salaris* may be unable to keep the parasite attached to the host skin during osmotic stress. Other studies have shown that cell and tissue functions can be inhibited when marine invertebrates are held in a hypoosmotic environment (Lange 1964; Pierce and Greenberg 1972; Prior and Pierce 1981). Hahnenkamp and Fyhn (1985) suggested that salmon lice might fall off their hosts in a hypoosmotic environment because of failing nerve and muscle functions. During hyperosmotic stress the cell and tissue functions of *G. salaris* are probably inhibited, as it was found that the parasites became opaque and immobile before death in 33.0‰. The function of the opisthaptor of *G. salaris* is dependent on muscular contractions (Malmberg 1957), and in cases of failing nerve and muscle functions it is likely that the parasite might be dislodged. Because *G. salaris* is an obligate parasite throughout its life cycle (Malmberg 1957), loose specimens die after dislodgement if not reattached to a new host. The survival time for unattached *G. salaris* in fresh water is dependent on temperature (maximum 132, 96, 54, and 24 h at 3, 7, 13, and 19°C, respectively; Mo 1987).

The present results support the hypothesis of brackish-water dispersal of *G. salaris* (Anonymous 1982; Johnsen and

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Jensen 1986; Lund and Heggberget 1992). Although not a euryhaline species, the salinity tolerance of *G. salaris* is found to be higher than previously reported and negatively correlated with temperature. However, the present laboratory experiments cannot be directly extrapolated to migrating fish in natural environments. Further work on the potential of *G. salaris* to remain attached to migrating hosts under salinity stress, undergo transmission in brackish water, reproduce in fresh water after saltwater exposure, and the functional hosts' potential for migration between river systems through estuaries and fjords are all important (cf. Soleng et al. 1997).

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Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill

Gary D. Marty, J. E. Hose, Michael D. McGurk, Evelyn D. Brown, and David E. Hinton

Abstract: Following the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, USA, Pacific herring (*Clupea pallasi*) larvae sampled from oiled sites had ascites, pericardial edema, and genotoxic damage. Laboratory study confirmed that these lesions were consistent with oil exposure. Pacific herring larvae were trawled from two oiled and two unoiled sites in Prince William Sound in May 1989. Larvae from oiled sites were shorter, had ingested less food, had slower growth (oiled, 0.07–0.10 mm/day; unoiled, 0.15–0.18 mm/day), and had higher prevalence of cytogenetic damage (oiled, 56–84%; unoiled, 32–40%) and ascites (oiled, 16%; unoiled, 1%) than from unoiled sites. In the laboratory experiment, Pacific herring eggs were exposed to an oil–water dispersion of Prudhoe Bay crude oil (initial concentrations of 0.0, 0.10, 0.24, 0.48, and 2.41 mg/L) and sampled for histopathology <24 h after hatching. Effects were significant at the 0.48 mg/L dose (Dunnett's procedure, $P < 0.05$). Lesions included ascites; hepatocellular vacuolar change; and degeneration or necrosis of skeletal myocytes, retinal cells, and developing brain cells. Lesions in field-sampled larvae were consistent with higher mortality rates documented in larvae from oiled sites.

Résumé : Après le déversement pétrolier de l'*Exxon Valdez* qui a eu lieu en 1989 dans le golfe du Prince-William, Alaska, É.-U., des larves de hareng du Pacifique (*Clupea pallasi*), prélevées dans des sites exposés au pétrole présentaient des ascites, de l'oedème péricardique et des lésions génotoxiques. Une étude en laboratoire a confirmé qu'il y avait compatibilité entre ces lésions et l'exposition au pétrole. Des larves de hareng du Pacifique ont été récoltées par chalutage dans deux sites exposés au pétrole et dans deux sites non exposés dans le golf du Prince-William en mai 1989. Les larves provenant des sites exposés étaient plus courtes, avaient ingéré moins d'aliments, avaient une croissance plus lente (site exposé, 0,07–0,10 mm/jour; site non exposé, 0,15–0,18 mm/jour) et présentaient une fréquence plus élevée de lésions cytogénétiques (site exposé, 56–84 %; site non exposé, 32–40 %) et d'ascites (site exposé, 16 %; site non exposé, 1 %) que les larves provenant des sites non exposés au pétrole. Au cours d'une expérience de laboratoire, des oeufs de hareng du Pacifique ont été exposés à une dispersion pétrole–eau de pétrole brut de Prudhoe Bay (concentrations initiales de 0,0, 0,10, 0,24, 0,48 et 2,41 mg/L) et ont été examinés pour déceler des manifestations histopathologiques moins de 24 h après leur éclosion. Les effets étaient statistiquement significatifs à la dose d'exposition de 0,48 mg/L (méthode de Dunnett, $P < 0,05$). Parmi les lésions figuraient des ascites, des modifications vacuolaires hépatocellulaires et une dégénérescence ou une nécrose des myocytes squelettiques, des cellules rétiniennes et des cellules cérébrales en développement. Les lésions chez les larves échantillonnées sur le terrain correspondaient avec les taux de mortalité élevés documentés chez les larves provenant des sites exposés au pétrole. [Traduit par la Rédaction]

Introduction

Fish embryos and larvae are the most sensitive life-history stage to toxicant exposure (Weis and Weis 1989). When the *Exxon Valdez* oil spill occurred in Prince William Sound (PWS), Alaska, U.S.A., in late March 1989, Pacific herring (*Clupea pallasi*) were beginning to congregate in shallow bays for their annual mass spawning in April. Previous studies had shown that Pacific herring embryos and larvae were susceptible to low concentrations of petroleum hydrocarbons (Smith and Cameron 1979; Cameron and Smith 1980; Pearson et al. 1985; Carls 1987), but oil-related damage had not been demonstrated

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G.D. Marty[1] and D.E. Hinton. Department of Anatomy, Phisiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA, 95616, U.S.A.
J.E. Hose. Occidental College, Biology Department, Los Angeles, CA, 90041, U.S.A.
M.D. McGurk. Triton Environmental Consultants Ltd., 120–13511 Commerce Parkway, Richmond, BC V6V 2L1, Canada.
E.D. Brown. Institute of Marine Science, School of Fisheries and Ocean Science, University of Alaska, Fairbanks, AK 99775-7220, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: gdmarty@ucdavis.edu

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under field conditions. Because contamination of Pacific herring embryos with *Exxon Valdez* oil seemed likely, several studies were initiated to determine effects of the spill on embryonic and larval development.

In two experiments, Pacific herring eggs were removed from oiled and unoiled sites in PWS and incubated in clean water in the laboratory. In one experiment, newly hatched larvae had oil-related cytogenetic damage and morphologic defects but no histologic lesions (Hose et al. 1996). In the other experiment, newly hatched larvae had no oil-related morphologic lesions, but cytogenetic and histopathologic analysis were not done (Pearson et al. 1995). In a laboratory experiment, Pacific herring eggs were exposed to an oil–water dispersion from fertilization to hatch, and newly hatched larvae were sampled for examination (Kocan et al. 1996). Significant oil-related and dose-dependent changes included premature hatching, low weight, and genotoxicity. These three experiments were limited in their ability to detect oil-induced damage in the 1989 year-class because exposure was terminated before hatch or immediately after hatch. In PWS, however, Pacific herring were potentially exposed to petroleum hydrocarbons throughout embryonic and larval development. Therefore, Pacific herring larvae were trawled from the nearshore environment in oiled and unoiled sites during May and early June of 1989. Estimated larval mortality in oiled areas was two times higher than in unoiled areas (McGurk and Brown 1996), but without more detailed study, increased mortality could not be definitively attributed to oil exposure.

In this paper, trawled larvae from McGurk and Brown (1996) were examined for cytogenetic and histologic lesions, and laboratory-exposed larvae from Kocan et al. (1996) were examined for histologic lesions. Significant histopathologic lesions in fish larvae potentially exposed to an oil spill have not previously been reported. We describe ascites as the most important lesion in larvae from oiled sites in PWS and confirm through laboratory study that this lesion can be caused by exposure to petroleum hydrocarbons. We also link oil exposure to delayed development in field-exposed larvae and to premature hatching of laboratory-exposed fish.

Methods

Environmental oil exposure

Pacific herring larvae examined from PWS were a subset of the larvae analyzed for growth and mortality by McGurk and Brown (1996). To summarize, Pacific herring larvae were captured using plankton trawls in two oiled areas and two unoiled areas in PWS. The designation of sites as oiled or unoiled was based on visual evidence of shoreline oil and the presence of polynuclear aromatic hydrocarbons characteristic of *Exxon Valdez* oil in intertidal mussels (Brown et al. 1996). Mean hatch date was estimated by McGurk and Brown (1996) for each site:

(1) Fairmont Bay, unoiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 3;

(2) Tatitlek Narrows unoiled site, sampled on May 11 and 21, 1989; estimated hatch date, May 4;

(3) Bass Harbor oiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 8; and

(4) Rocky Bay oiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 10.

Spawning occurred at all sites between April 8 and 15; oiled sites were about 1°C colder, 1‰ more saline, and the water column was more mixed than control stations (McGurk and Brown 1996).

Larvae were preserved in 5% seawater formalin (1 part 37% formaldehyde diluted into 19 parts undiluted seawater taken from the sea surface at the side of the boat), and 25 larvae from each site and each date (200 total) were randomly selected for gross morphology, cytogenetics, and histopathology. Craniofacial and finfold abnormalities were evaluated using methods described in Hose et al. (1996). Graduated severity index scores assigned to each variable reflected no defect (score = 0), mild defect in structure or size (score = 1), moderate defect in structure or size (score = 2), or severe or multiple defect(s) in structure or size (score = 3). A pectoral fin was removed and evaluated for cytogenetic abnormalities using Hose et al.'s (1996) method of enumerating the numbers of mitotic cells and apoptotic (pyknotic) cells per fin. Prevalences of cytologically abnormal fins (those with abnormal cells) and cytogenetically abnormal fins (high anaphase aberration rate or low mitotic rate) were determined.

McGurk and Brown (1996) estimated growth from body length measurements of Pacific herring larvae captured from at least five cruises at each site from May 3 through June 12, 1989. To evaluate whether larvae from the two cruises per site used in this study had significant differences in growth not detected in the broader study, body length was measured independently of McGurk and Brown (1996) using an Olympus Cue-2 Image Analyzer. Growth at each site was estimated by subtracting the mean length of the first sample from the mean length of the second sample and dividing by the number of days between samples. Methods for histopathology were described by Hose et al. (1996), except that all larvae for histopathology were embedded in glycol methacrylate (GMA) instead of paraffin. Each GMA-embedded larva was serially sectioned at 5 µm, and sections were saved at 20-µm intervals so that between 12 and 30 sections of each larva were examined.

For histopathologic examination, sections of each larva were scanned at 100× magnification for major organs: gonad, retina, brain, heart, branchial arch, skin, skeletal muscle, kidney, gastrointestinal tract, yolk sac, liver, and spleen. Gonads were undifferentiated and gender could not be determined. The extent of liver glycogen was ranked and scored as minimum (no obvious hepatocellular clear, pale areas; score = 1), moderate (extent of hepatocellular clear, pale areas less than nuclear area; score = 2), or abundant (volume of hepatocellular clear, pale areas greater than nuclear area; score = 3). Yolk and the surrounding yolk sac were classified as abundant (sectional area of brightly eosinophilic, homogeneous, proteinaceous yolk was greater than the sectional area of the surrounding yolk sac cells; score = 3), moderate (sectional area of yolk less than yolk sac; score = 2), minimal (only the weakly staining yolk sac remained; score = 1), or absent (no yolk or yolk sac; score = 0).

Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Lesions included (1) myodegeneration and necrosis; (2) hepatic vacuolar change; (3) individual hepatocellular necrosis; (4) lens vacuolation; (5) retinal degeneration or necrosis; (6) brain degeneration or necrosis, (7) proteinaceous ascites; and (8) histiocytic peritonitis. We used "ascites" to describe accumulation of fluid around viscera within the body cavity; the lesion is morphologically the same as described by Spitsbergen et al. (1991) as "subcutaneous edema" (J.M. Spitsbergen, Oregon State University, Corvallis, OR 97331, personal communication). For quality control, each larva was examined for extent of autolysis and tissue artifact and scored using the same semiquantitative scale. The relative amount of food in the gastrointestinal tract was scored as none (0), minimal (1), moderate (2), or abundant (3).

Laboratory oil exposure

Laboratory-exposed Pacific herring larvae were a subset of the larvae analyzed for hatching, morphologic abnormalities, and genotoxicity by Kocan et al. (1996). To summarize, Pacific herring eggs were continuously exposed to an oil–water dispersion (OWD) of *Exxon Valdez* crude oil. Initial concentrations of high molecular weight (C12–C28) diesel range hydrocarbons were 0, 0.1, 0.24, 0.48, 0.97,

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Table 1. Multiple analysis of variance (MANOVA) on histopathology and cytogenetic scores in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill.

Effect or relation	Significant principal component (most important lesion scores)[a]	Level of significance
	Histopathology scores	
Oil	Overall comparison (MANOVA)	***
Oil	First (yolk, gastrointestinal food, tissue artifact, ascites)[a]	***
Oil	Third (hepatocellular degeneration, vacuolar change, and glycogen; gastrointestinal food)	***
Site (oil)	Overall comparison (MANOVA)	***
Site (oil)	Third (hepatocellular degeneration, vacuolar change, and glycogen; gastrointestinal food)	**
Site (oil)	Fourth (histiocytic peritonitis, myodegeneration and necrosis, tissue artifact)	***
	Cytogenetic scores and length	
Oil	Overall comparison (MANOVA)	***
Oil	First (cytogenetically abnormal, craniofacial malformations, number of mitotic figures per fin, fin fold malformations, body length)	***
Oil	Second (number of apoptotic cells per fin, cytologically abnormal)	*
Site (oil)	Overall comparison (MANOVA)	ns

Note: Analysis tested the influence of exposure status (oil) and the influence of site as a nested factor within exposure status (site (oil)). Because of missing values, 183 of 200 fish were used to analyze histopathology scores, and 158 of 200 fish were used to analyze cytogenetic scores. Significances are as follows: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Overall comparisons (MANOVA) did not depend on individual principal components. Principal components not shown were not significant.

[a]The first principal component contributes most to variability, whereas the second and later principal components contribute progressively less to variability. Within a significant principal component, lesions are listed in descending order of importance.

and 2.41 mg/L (100% OWD = 9.67 mg/L; Kocan et al. 1996). Larvae were sampled immediately after hatch to allow direct comparisons with other postspill field studies in which sampling occurred within 24 h of hatching (Pearson et al. 1995; Hose et al. 1996). Because laboratory techniques for feeding larvae had not been developed at the time of the experiment, larvae were not held beyond 24 h posthatch; therefore, same-age comparisons of laboratory-exposed larvae with field trawls of planktonic larvae were not done. Every 24 h, all larvae in each container (i.e., those that had hatched in the previous 24 h) were anesthetized in tricaine methanesulfonate and transferred to 5% neutral buffered formalin. Later, fixed larvae were checked for abnormalities under a dissecting microscope, and a pectoral fin was removed for cytogenetic analysis (Kocan et al. 1996). Three groups of larvae were analyzed for histopathologic lesions:

(1) random selection, paraffin embedment (50 larvae): 10 control larvae and 10 larvae each from the 0.10, 0.24, 0.48, and 2.41 mg/L OWD exposures;

(2) nonrandom selection, paraffin embedment (22 larvae): five larvae each from the 0.10, 0.24, 0.48, and 2.41 mg/L OWD exposures and two larvae from the 0.97 mg/L OWD exposure; and

(3) nonrandom selection, GMA embedment (16 larvae): seven control larvae and two or three larvae at each of the 0.24, 0.48, 0.97, and 2.41 mg/L OWD exposures.

Nonrandom samples were taken to determine the histologic features of observations made with the dissecting microscope; results from nonrandom samples were not used for statistical analysis. Because fish reared in the highest OWD doses hatched earlier, age at sampling (days postfertilization) decreased with dose: controls (19–20 days old); 0.10 and 0.24 mg/L (19 days old); 0.48 mg/L (16–17 days old); and 2.41 mg/L (14–15 days old).

All 88 larvae were assigned a unique random number for further processing and blind study. For paraffin-embedded larvae, groups of three or four serial sections were saved at approximately 25-μm intervals through each larva. Larvae embedded in GMA were processed the same as larvae sampled from PWS. Nearly all paraffin sections had moderate artifact, but section quality of the GMA-embedded larvae was superior.

Statistical analysis

Results from the field and laboratory experiments were analyzed separately using similar methods. Lesion scores were first summarized through use of a principal components analysis (Ehrenberg 1975). For a given set of scores, the first four principal components were used as a summary for most of the variability in the raw data. The principal component factor scores were then tested for systematic effects of oil exposure using a multivariate analysis of variance (MANOVA; Johnson and Wichern 1992). After the MANOVA, univariate ANOVA models were run on each of the individual principal components (Winer et al. 1991). In the field study, sample site was treated as a nested factor within oiled status to separate out differences between sites that might be unrelated to oil exposure. To compare histologic changes, Spearman's rank correlation coefficients were calculated on eigenvalues derived from principal components analysis. Cytogenetic results were analyzed separately from histopathology scores. In the laboratory experiment, Dunnett's comparison was used to test each exposure group against the control, and Spearman's correlation coefficients were calculated to compare OWD dose to histologic changes. Comparisons in both experiments were categorized as not significant ($P > 0.05$), significant ($P \leq 0.05$), or highly significant ($P \leq 0.01$). To demonstrate trends in the data, mean scores (±SE) were graphed for all lesions. Because the probability of type-I error increases with multiple analyses, ANOVA was not done on scores for individual lesions.

Results

Environmental oil exposure

Pacific herring larvae from oiled sites had cytogenetic and histopathologic lesions that were highly significant when compared with larvae from unoiled sites, and several variables contributed to the differences (Table 1). Site as a nested factor within exposure group was highly significant for histopathology but not cytogenetic scores.

Ascites was the most consistent lesion associated with oil exposure, affecting 16 of 100 larvae from oiled bays but only 1 of 97 larvae from unoiled sites. In fish with ascites, the peritoneal cavity was distended by pale eosinophilic fluid that sometimes also expanded the pericardial space (pericardial edema or hydropericardium; Fig. 1A). By comparison, normal larvae had minimal clear pericardial fluid (Fig. 1B). All fish with pericardial edema also had ascites. Fish with ascites usually had abundant yolk, and yolk scores were correlated with scores for ascites (Spearman's correlation, $r^2 = 0.30$, $P = 0.0001$).

In addition to ascites, larvae from oiled sites had several histologic and cytogenetic changes consistent with delayed

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Fig. 1. Micrographs of Pacific herring larvae. Fish are oriented with the head to the left; hematoxylin and eosin stain. (A) Parasagittal section of the anterior half of a larva from Bass Harbor (oiled site) with severe ascites (a) around abundant yolk (y), and moderate pericardial edema surrounding the heart (h). Scale bar = 200 µm. (B) Parasagittal section of a normal larva from Bass Harbor (oiled site) in which the heart (h) is surrounded by minimal clear fluid (f). Scale bar = 150 µm. (C) Mild histiocytic peritonitis in a larva from Tatitlek Narrows (unoiled site). Scattered histiocytes (large arrow) partly surround the resorbing yolk sac epithelium (small arrows), which is elongate and contains multiple, large, basophilic, condensed nuclei. Scale bar = 80 µm. (D) Foregut of a larva exposed to oil in the laboratory contains large numbers of bacteria (arrows) surrounded by degenerating cells; note homogeneous proteinaceous ascites ventral to the foregut and surrounding the scale bar. Scale bar = 150 µm. (E) Liver with no hepatocellular cytoplasmic vacuoles. Scale bar = 30 µm. (F) Liver with mild hepatocellular cytoplasmic vacuoles; magnification same as (E).

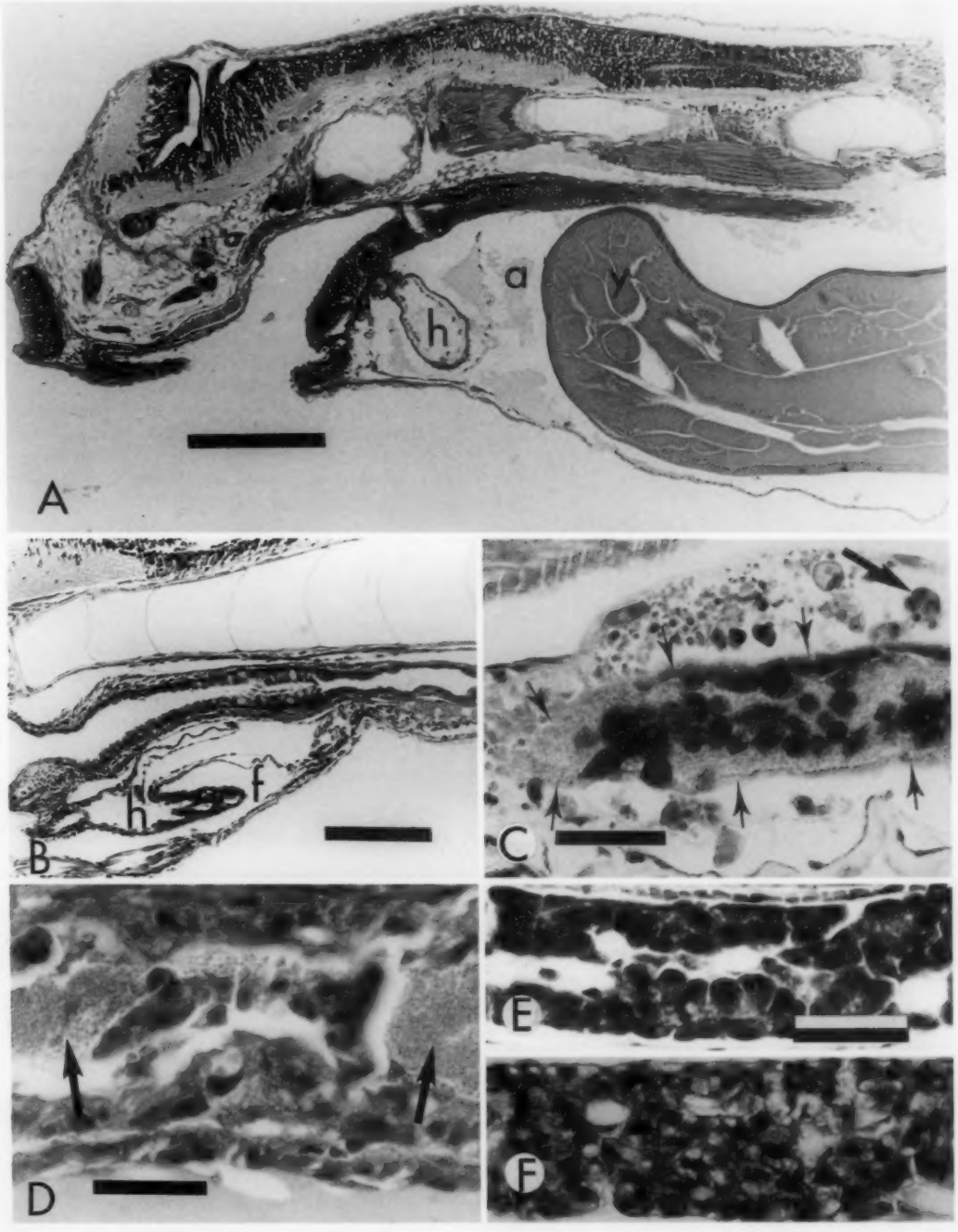

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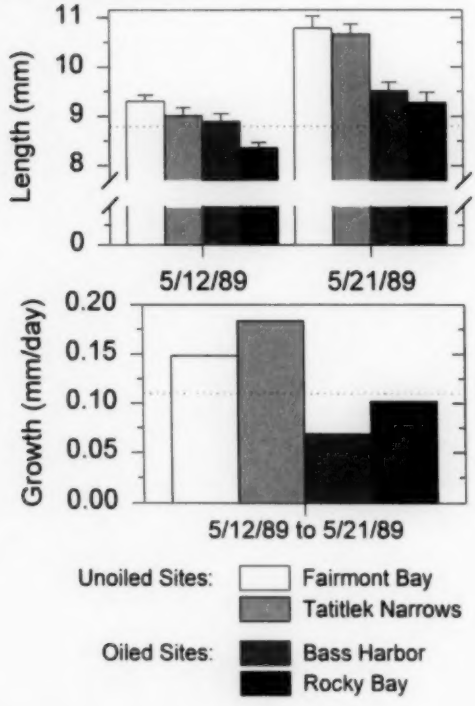
Fig. 2. Mean total length and estimated growth of Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. The reference line in the top graph is at 8.8 mm, the estimated length at hatch for these larvae (McGurk and Brown 1996); the reference line in bottom graph highlights growth differences between unoiled and oiled sites. Error bars are 1 SE.



development and growth (Figs. 2–4). Fish from oiled sites were shorter and grew slower than fish from unoiled sites (Fig. 2). Sectional area of the yolk sac was used as the best histologic marker of development. As expected, mean yolk scores decreased from the first to the last sample in larvae from all sites. However, yolk scores were consistently greater in fish from oiled sites (Fig. 3). Likewise, the amount of food in the gastrointestinal tract increased during the study, but the increase was less in larvae from oiled sites. Larvae from Rocky Bay never had food in their gastrointestinal tract (Fig. 3). Digestion of food in the gastrointestinal tract usually was minimal, and identifiable food consisted of eggs, copepod larvae, and an unidentified metazoan (probably a decapod) that had abundant striated muscle (Fig. 4). Three of the 61 larvae with moderate to abundant yolk also had food material in their gastrointestinal tract; two were from an oiled site and one was from an unoiled site. As larvae increased in size, hepatocellular glycogen and cytoplasmic vacuolation increased. Hepatocellular glycogen was minimal in all larvae from the first set of samples (Fig. 1E). In the last set of samples, most larvae had minimal glycogen, but four fish from unoiled sites had moderate amounts of glycogen. Cytoplasmic vacuoles in hepatocytes were multiple, fairly well demarcated, and 2–10 μm in diameter. Vacuole morphology was most consistent with lipid (Fig. 1F), but the presence of lipid could not be confirmed because lipids were lost during tissue processing. Although cytoplasmic vacuoles were significantly correlated with increasing length, they were not significantly correlated with increasing scores for gastrointestinal food.

Histiocytic peritonitis was the other development-associated histologic change that was delayed in fish from oiled sites. Histiocytic peritonitis occurred when yolk stores were depleted and the yolk sac epithelium was being resorbed through a process of apoptosis, or programmed cell death (score = 1, Fig. 3). The resorbing yolk sac epithelium was often surrounded by small numbers of foamy macrophages (histiocytes), producing a mild peritonitis (Fig. 1C). Histiocytic peritonitis was absent from larvae with abundant yolk and in larvae that had completed yolk sac absorption.

Myodegeneration and necrosis was primarily a site effect (Table 1). The lesion was more frequent and severe in fish from the last set of samples, and scores were greatest for fish from Tatitlek Narrows (Fig. 3). Degenerating myofibers had clear, irregular, cytoplasmic vacuoles, hypereosinophilic cytoplasm, and pyknotic nuclei (Fig. 5B).

Among morphologic, cytologic, and cytogenetic changes, oil-related differences were most significant for cytogenetic and morphologic changes (Table 1, Fig. 6). Finfold and craniofacial lesions occurred in all samples, but mean scores were consistently greater in fish from oiled sites (Fig. 6). Prevalence of cytologic lesions contributed significantly to oil-related differences (Table 1), but differences in prevalence of cytologic lesions were less consistent than morphologic changes. All samples had some fish with cytogenetic damage, anaphase aberrations were more prevalent in fish from oiled sites, regardless of developmental stage (Fig. 6). Among other subcellular changes, increased numbers of mitotic figures and apoptotic cells in fish from unoiled sites were consistent with increased growth rates in larvae from unoiled sites.

Laboratory oil exposure

As in field samples, ascites was the most consistent and sensitive oil-related lesion in larvae exposed to oil during development in the laboratory. A high proportion of larvae were affected at initial doses as low as 0.24 mg/L (Fig. 7), although the lowest dose resulting in significant overall effects was 0.48 mg/L. Ascites prevalence was only 10% for the control and 0.1 mg/L treatments, 60% in each of the 0.24 and 0.48 mg/L treatments, and 100% in the 2.41 mg/L treatment. Ascites was more highly correlated with oil dose than any other variable (Table 2).

Several other variables contributed to highly significant oil-related differences, and increases in mean scores for significant lesions were dose dependent (Table 3, Fig. 7). The second variable most highly correlated with oil dose was hepatocellular vacuolar change (Table 2); no control fish had this lesion (Fig. 7). Hepatocytes in affected larvae had irregular, poorly demarcated, cytoplasmic vacuoles, and nuclei often were pyknotic. Vacuole morphology was consistent more with hydropic degeneration (fluid accumulation) than with the morphology of lipid vacuoles in hepatocytes of field-sampled fish. Most laboratory-exposed fish with vacuolar change also had moderate autolysis.

Autolysis was most common in larvae from the two highest doses (Fig. 7), and its correlation with oil dose was highly significant (Table 2). However, autolysis was not among the

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Fig. 3. Significant histopathology scores in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. Scales vary, but the reference line score is the same in every graph. Error bars are 1 SE.

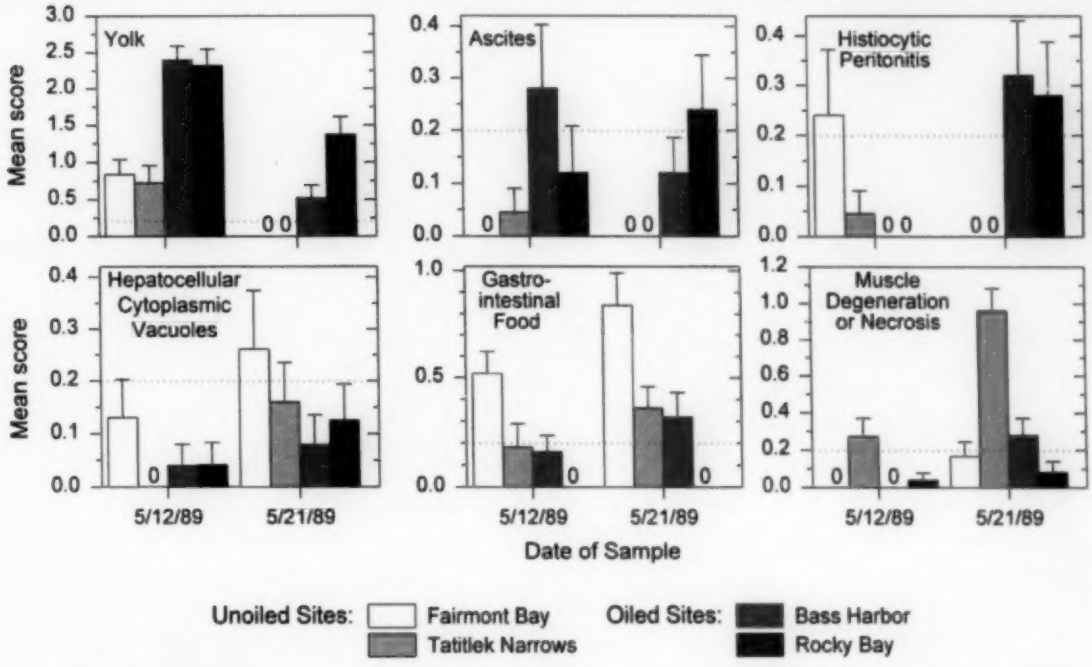

Fig. 4. Minimally digested food in the intestinal tract of Pacific herring larvae from Fairmont Bay (unoiled site), hematoxylin and eosin stain. (A) Four organisms (probably larvae of a mollusc). Scale bar = 150 μm. (B) Two eggs. Scale bar = 50 μm. (C) A probable copepod nauplius. Scale bar = 50 μm.

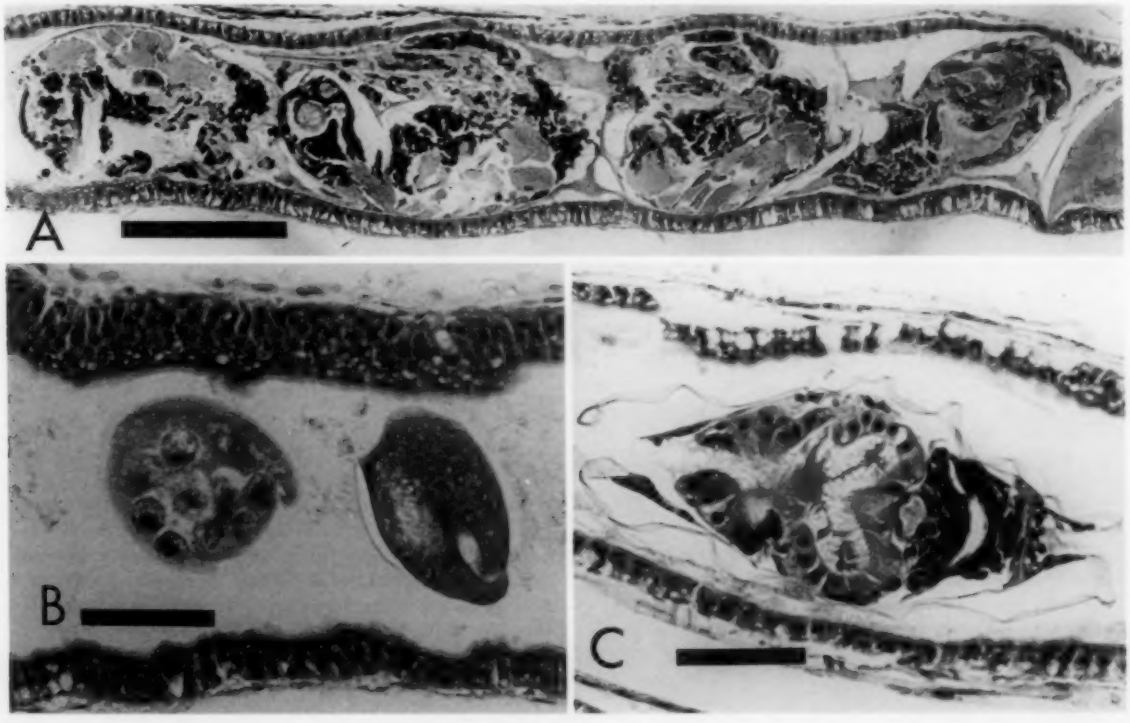

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Fig. 5. Epaxial muscle of Pacific herring larvae, hematoxylin and eosin stain. Scale bar = 80 μm. (A) Normal skeletal muscle fibers were parallel and not vacuolated. (B) Degenerative and necrotic myofibers (arrows) were irregular, with cytoplasmic vacuoles and pyknotic nuclei (larva was exposed oil in the laboratory).

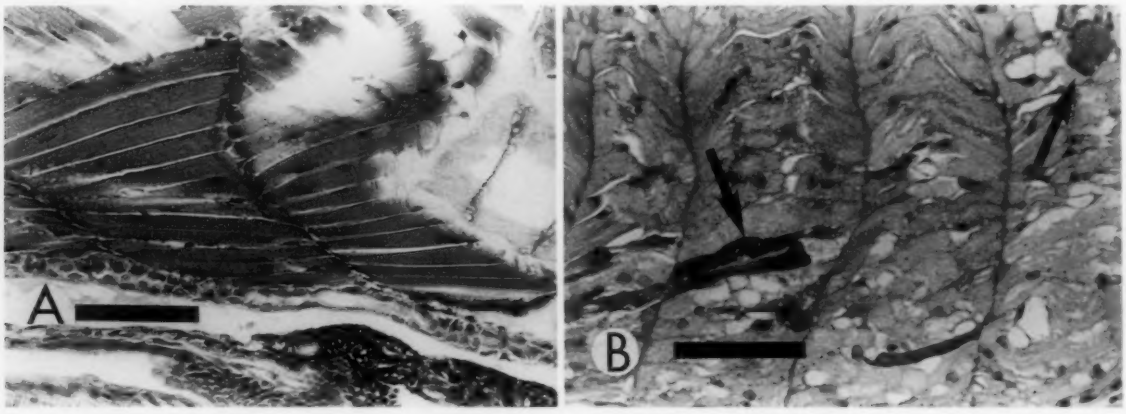
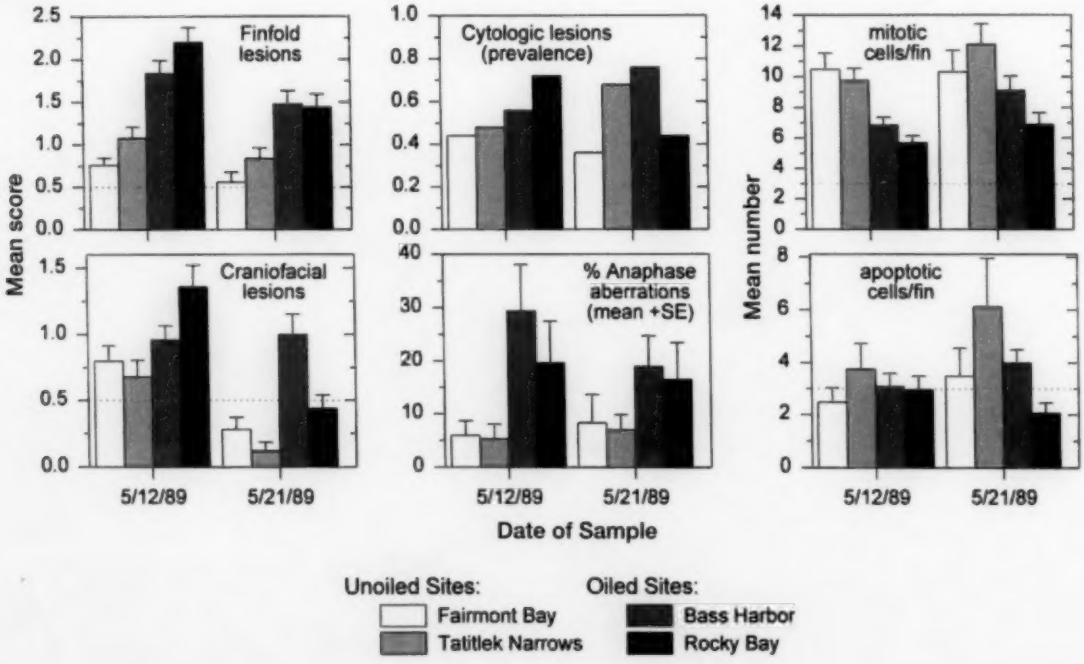


Fig. 6. Significant gross, cytologic, and cytogenetic results in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. Scales vary, but the reference line value is the same within each column of graphs. Error bars are 1 SE.



three lesions that contributed most to variability within significant principal components (Table 3). Larvae that were partly autolyzed often had bacterial colonies in the digestive tract, and colonies were occasionally infiltrated by macrophages or surrounded by degenerating cells (Fig. 1D).

Other significant lesions included degeneration and necrosis of skeletal myocytes, retinal cells, and brain cells (Table 3). Myodegeneration and necrosis was similar to that described in field-sampled larvae (Fig. 5). Degeneration and necrosis within the retina and brain were uncommon. In affected foci, individual cells or small clusters of cells had pyknotic nuclei and condensed hypereosinophilic cytoplasm. Lens vacuolation was the same as described by Hose et al. (1996), and all six larvae with lens vacuolation were moderately or severely autolyzed. Also, among the 38 fish selected nonrandomly for examination, 11 had lens vacuolation, and all but one of these was moderately or severely autolyzed.

Oil-related changes associated with delayed development were not as pronounced in these larvae as they were in field-sampled larvae. Instead, lesions in exposed fish were

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Fig. 7. Histopathology scores from Pacific herring larvae exposed to an oil–water dispersion during embryonic development. Ten larvae were examined from each dose. The reference line is included to clarify differences in bar length. Error bars are 1 SE.

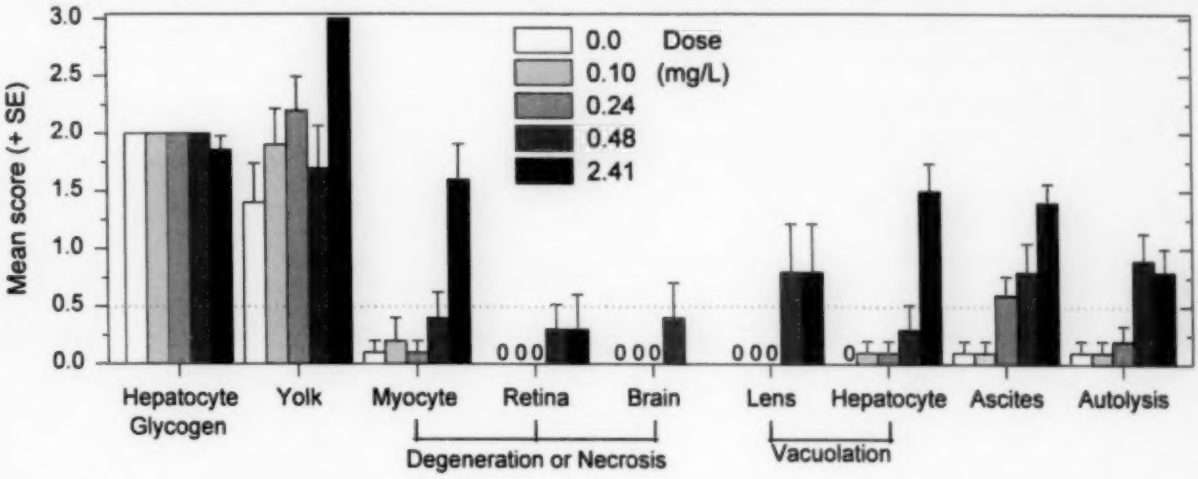

Table 2. Correlation between scored variables (lesions) and oil dose in newly hatched Pacific herring larvae exposed to an oil–water dispersion during embryonic development.

Scored variable (lesion)	r^2	Significance
Ascites	0.68[a]	***[b]
Hepatocellular cytoplasmic vacuoles	0.58	***
Autolysis	0.53	***
Myodegeneration or necrosis	0.53	***
Yolk	0.43	***
Lens vacuolation	0.39	***
Retinal degeneration or necrosis	0.24	ns
Hepatocellular glycogen	–0.22	ns
Brain degeneration and necrosis	0.14	ns

[a]Spearman correlation coefficients on eigenvalues derived from principal components analysis (n = 47–50 for each determination).

[b]Significances are as follows: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Table 3. Multiple analysis of variance (MANOVA) on histopathology scores in Pacific herring larvae exposed to an oil–water dispersion of petroleum hydrocarbons during early development in the laboratory.

Effect or relation	Significant principal component (and most important lesion scores)[a]	Level of significance
Oil (categorical)	Overall comparison (MANOVA)	***
Oil (categorical)	First (hepatocellular vacuolar change, ascites, and myodegeneration or necrosis)[a]	***
Oil (categorical)	Second (hepatocellular glycogen, retinal degeneration or necrosis, and myodegeneration or necrosis)	*
Oil (linear)	Overall comparison (MANOVA)	***
Oil (linear)	First (hepatocellular vacuolar change, ascites, and myodegeneration or necrosis)	***
Oil (linear)	Third (lens vacuolation, brain degeneration, or necrosis)	*

Note: Analysis tested the influence of oil exposure as a categorical effect or as a linear effect. Because of missing values, 43 of 50 fish were used in the analysis. Significances are as follows: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Overall comparisons (MANOVA) did not depend on individual principal components. Principal components not shown were not significant.

[a]The first principal component contributes most to variability, whereas the second and later principal components contribute progressively less to variability. Within a significant principal component, lesions are listed in descending order of importance.

consistent with premature hatching. For example, yolk reserves tended to increase with dose, and differences were most prominent between the control and high-dose groups (Fig. 7). All exposure groups had moderate amounts of hepatocellular vacuolation attributed to glycogen stores, but one high-dose fish had minimal hepatocellular glycogen (Fig. 7). In general, the distribution of lesions in the 38 nonrandomly selected larvae was the same as in the randomly selected larvae, with dose-dependent increases in ascites, hepatocellular vacuolar change, and all degenerative and necrotic lesions compared in Fig. 7.

Discussion

In both field and laboratory experiments, ascites was the most significant lesion related to oil exposure. Ascites was clearly related to oil exposure under controlled conditions in the laboratory, but because of variable environmental conditions, the link between ascites and oil exposure in field-sampled larvae was less clear. Were hydrocarbon concentrations in PWS high enough to explain the development of ascites? Several studies have reported toxic effects of the water soluble fraction of crude oil on Pacific herring embryonic development, and levels required to produce toxicity range from 0.1 to 1.0 mg/L (Smith and Cameron 1979; Cameron and Smith 1980; Kocan et al. 1996). In PWS after the oil spill, concentrations of polynuclear aromatic hydrocarbons (PAH) in surface water (depth up to 5 cm) from oiled sites averaged 0.9 µg/L in late April and early May 1989 (Neff and Stubblefield 1995); PAH

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concentrations at a depth of 1 m were 0.5 to 1.0 µg/L in mid-April, but at those depths, PAHs were detectable only near heavily oiled beaches by early May (Short and Harris 1996). Neff and Stubblefield (1995) used water samples from PWS in 1989 to perform standard acute and chronic toxicity tests on three species of marine animals. Based on previously published values and lack of toxicity in their samples, they concluded that "it is likely that the safety factor (ratio of effects concentration to environmental concentration) for marine organisms in PWS following the spill was in excess of 100 and possibly as high as 10 000 during April and May 1989" (Neff and Stubblefield 1995).

However, recent laboratory studies provide new evidence that indigenous fish species are sensitive to PAH concentrations of 0.2–5 µg/L when exposure continues throughout embryonic and early larval development. In these studies, ascites was one of the most sensitive markers of PAH exposure. Pink salmon (*Oncorhynchus gorbuscha*) incubated in gravel substrate were exposed from fertilization through emergence to an initial PAH concentration of 31.8 µg/L that decreased to less than 3 µg/L after day 50 of incubation. Ascites developed in 2.6% of exposed fish (114 of 4380), but none of 3722 fish from control incubators developed ascites (Marty et al. 1997). In Pacific herring exposed to PAH from fertilization to hatching, 20% of larvae developed ascites after exposure to 0.3 µg/L PAH, and 0.7 µg/L PAH was sufficient to cause ascites in 50% of exposed fish (M.G. Carls, U.S. National Marine Fisheries Service, Auke Bay, Alaska; personal communication). Therefore, ascites prevalence of 16% in Pacific herring larvae from oiled sites in PWS is consistent with continuous laboratory exposure to less than 0.3 µg/L PAH: concentrations well within documented PAH levels in PWS in April and May 1989 (Neff and Stubblefield 1995; Short and Harris 1996). Increased sensitivity in these recent studies provides strong evidence that toxicity of multiring PAHs is greater than the toxicity of low molecular weight hydrocarbons that predominate in water-soluble fractions or oil–water dispersions.

Within each experiment, affected larvae had several lesions other than ascites. From oiled sites in PWS, several lesions were associated with delayed or prolonged development. By comparison, larvae exposed to oil in the laboratory had several degenerative or necrotic lesions, but developmental differences were less distinct and related to premature hatching. Skeletal myodegeneration or necrosis in laboratory-exposed fish was most common after exposure to the highest oil dose but, in field samples, was most common at a reference site; therefore, myodegeneration in larvae from PWS cannot be exclusively attributed to oil exposure.

Hydrocarbon exposure was the most likely cause of ascites and pericardial edema in our studies. Because these lesions are not specific for hydrocarbon exposure, other causes of ascites must be ruled out. Atlantic herring (*Clupea harengus*) larvae reared in hypotonic (7‰) or hypertonic (35‰) salt water developed pericardial edema and ascites (Dushkina 1973). However, all measured or extrapolated salinities at sample sites were greater than 25‰ (McGurk and Brown 1996, Fig. 2), and the chance that larvae were exposed to salinities <10‰ was minimal. Pericardial edema has been described in many developmental studies with several fish species and different toxicants (Waterman 1940; Weis and Weis 1974; Dial 1978; Gillespie and Baumann 1986; Middaugh et al. 1988; Marty et al. 1990), but ascites is less often described. Ascites was the main lesion associated with blue-sac disease in wild lake trout (*Salvelinus namaycush*) larvae, and ascites also occurred in lake trout larvae exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs (Symula et al. 1990; Spitsbergen et al. 1991). Despite detailed study of water samples from PWS, no toxicants other than oil-related PAH were identified that could explain the high prevalence of ascites in fish from oiled sites in PWS. In addition to physical and chemical causes of ascites, biological causes must also be considered. Viral hemorrhagic septicemia virus has recently been isolated from adult Pacific herring in several locations in Alaska, British Columbia, and Washington (Meyers et al. 1994; Meyers and Winton 1995), and related viruses cause ascites in young salmonids (Wolf 1988). Viral contribution to the pathogenesis of ascites in larval Pacific herring in 1989 cannot entirely be ruled out, but larvae lacked hemorrhagic lesions that have been associated with viral hemorrhagic septicemia in older fish (Meyers et al. 1994), and a viral cause of ascites seems unlikely.

Ascites and pericardial edema probably also contributed to the pathogenesis of delayed development and degenerative or necrotic lesions. Edema (and ascites) can develop by several mechanisms (Cotran et al. 1989), but the pathogenesis in this study was not determined. Regardless of the mechanism of edema formation, increased fluid pressure around the heart and major vessels probably restricted atrial stroke volume, leading to decreased venous return and subsequently, decreased blood flow to tissues. If blood flow to tissues decreased, then developing organs would have received fewer nutrients, resulting in decreased growth and development.

Decreased growth in larvae from oiled sites is consistent with findings in three other laboratory studies with Pacific herring. First, embryonic exposure to the water-soluble fraction of Prudhoe Bay crude oil for 48 h resulted in pericardial edema, decreased growth, and jaw lesions (Smith and Cameron 1979). Second, larval exposure to the water-soluble fraction of crude oil in food resulted in decreased growth (Carls 1987). In the same study, oil-contaminated prey (6 mg/L) caused larval mortality but did not affect feeding or growth of survivors. Thirdly, embryonic exposure to 45 mg/L benzene (an aromatic component of crude oil) resulted in delayed development, irregular heartbeat, and fin and jaw lesions, and larval exposure to 12 mg/L decreased feeding and growth (Struhsaker et al. 1974). Note that Carls (1987) found that the water-soluble fraction he used contained 95% monoaromatic and only 5% diaromatic hydrocarbons, so direct comparisons of total aromatic hydrocarbons in these laboratory studies with PAHs in the water column of PWS in 1989 are not possible.

Increased yolk volume in larvae from the highest laboratory exposure group was consistent with premature hatch. These larvae hatched up to 3 days earlier than controls but weighed significantly less (Kocan et al. 1996). Weight differences provided evidence that energy was going to oxidative activity rather than growth. Induction of cytochrome P450 enzymes has been hypothesized as a cause of increased metabolism in oil-exposed fish larvae (Moles et al. 1987), but specifics of this hypothesis have not been critically evaluated. Alternatively, cardiovascular dysfunction in the high-dose group might have increased anaerobic metabolism resulting in less efficient use of yolk stores. Oil-associated differences in yolk volume were

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more distinct in larvae from the field experiment, but the exact age of these fish was unknown and the possibility that younger fish were sampled from oiled sites cannot be eliminated. Water temperatures were consistently about 1°C lower at oiled sites, but this small difference probably was not enough to explain all differences in developmental rates between oiled and unoiled sites. Interestingly, feeding by fish larvae that still have yolk has been readily demonstrated in laboratory studies, but has been difficult to corroborate in field surveys (Heming and Buddington 1988). In our study, three Pacific herring larvae had gastrointestinal food and moderate to abundant yolk; these findings may have been enhanced by immediate fixation of whole larvae, embedment in GMA, and examination of parasagittal step sections.

In severe cases of pericardial edema and ascites, blood flow is sufficiently restricted that tissues become necrotic, eventually fish die, and tissues autolyze and are colonized by bacteria. In observations of living embryos and larvae (Waterman 1940; G.D. Marty and J.E. Hose, unpublished data), bacterial growth and necrosis can involve a large fraction of the body even when the rudimentary heart continues pulsatile movement; in these cases, blood flow is poor or has ceased. Hence, the exact time of death is often difficult to pinpoint in larval fish. In the laboratory study, colonies of bacteria probably grew after death in some larvae, but inflammatory cells admixed with the bacteria in these larvae were evidence that some bacterial growth occurred before death. Malformed Pacific herring embryos often die during hatching (R.M. Kocan, University of Washington, Seattle, WA 98195, personal communication). Larvae for the laboratory experiment were not closely examined under a microscope before being placed in fixative, and several larvae probably were dead before fixation. Some of these larvae might have been incorrectly been placed into the "% live hatch" category by Kocan et al. (1996); however, because most autolyzed larvae were also malformed, the "% normal" category as reported by Kocan et al. (1996) probably was minimally affected by postmortem changes. Problems differentiating necrosis from autolysis could be eliminated in future studies if all larvae slated for histopathology are examined for an active heart beat before they are preserved.

Lesions in Pacific herring larvae exposed to oil have also been described in lake trout larvae exposed to chlorinated dioxin during embryonic development (Spitsbergen et al. 1991). Lake trout larvae often died near the time of hatch, and survivors developed severe ascites and pericardial edema, resulting in cessation of blood circulation in the yolk sac and body (Spitsbergen et al. 1991). Necrosis of the retina, brain, and spinal cord occurred in morbid embryos and sac fry, and all larvae in the highest dose group had arrested development after hatching. In another study, surf smelt (*Hypomesus pretiosus*) embryos had necrotic neurons in the forebrain and neuronal layer of the retina after exposure to 54 or 113 µg/L water-soluble fraction of Cook Inlet crude oil for 15–21 days (Hawkes and Stehr 1982).

In the laboratory experiment, mean scores for ascites were greater in fish exposed to 0.24 mg/L than in controls, but overall increases in histopathologic lesions were not statistically significant until initial OWD concentrations reached 0.48 mg/L. Therefore, histopathology was less sensitive for detecting oil-related lesions than was cytogenetic analysis (0.10 mg/L) and equally as sensitive as examination for gross physical deformities (Kocan et al. 1996). In the field experiment, histopathologic, cytogenetic, and morphologic examination all yielded significant oil-related differences. Comparison of the two experiments is limited because of differences in age at sampling and probable differences in exposure, but prevalence and severity of lesions in larvae from oiled sites were roughly equivalent to lesions in larvae from the laboratory exposed to 0.24 mg/L. For example, both groups of fish had ascites and pericardial edema, but neither group had degenerative and necrotic lesions in the brain and retina. Eggs that were spawned in oiled sites in PWS in 1989, but later moved to clean water in the laboratory for incubation until hatching, had significant oil-related cytotoxicity but not ascites (Hose et al. 1996). The relatively high prevalence of autolysis in that experiment, and resultant rupture of the yolk sac, may have masked the identification of ascites, which requires an intact body wall. Alternatively, Pearson et al. (1995) did not observe oil-related differences in prevalence of pericardial edema, indicating that longer exposure might have been needed to cause ascites and other histologic lesions. Methods have recently been developed for long-term culture of Pacific herring larvae and juveniles in the laboratory (R.M. Kocan, personal communication), and additional laboratory studies are planned to determine the role of early oil exposure on long-term survival and growth.

In the field experiment, growth was an important component of significant oil-related effects in the subset of larvae examined histologically in this study, but oil-related effects on growth were not significant for estimates from a larger series of trawls in PWS (McGurk and Brown 1996). At least two explanations are possible. First, subsamples of larvae for histopathology were from a much smaller range (May 11–21) than were all larvae sampled by McGurk and Brown (May 3–30), and subtle differences in growth may have been significant only within the shorter range. Second, McGurk and Brown (1996) estimated growth based on the assumption that newly hatched larvae were 8.8 mm long. However, Kocan et al. (1996) found that oil-exposed eggs hatched earlier and had a lower dry weight than control fish; therefore, the assumption may be incorrect that Pacific herring throughout PWS were the same size at hatch. In juvenile pink salmon sampled from PWS after the *Exxon Valdez* oil spill, growth was less in oiled areas than in unoiled areas (Weidmer et al. 1996; Willette 1996), and oil-related decreases in growth were confirmed in the laboratory (Schwartz 1985; Carls et al. 1996). Juvenile Pacific herring were often caught with pink salmon in oiled sites in 1989 (Wertheimer and Celewycz 1996). It is, therefore, reasonable to conclude that estimated growth differences in Pacific herring sampled for histopathology were real. Also, lack of food material in any of 50 larvae examined from Rocky Bay is consistent with decreased growth in fish from this oiled site. In laboratory experiments, starved Atlantic herring larvae ceased to grow (Ehrlich et al. 1976); therefore, growth of Pacific herring from Rocky Bay (0.10 mm/day) is evidence that available food had been captured and moved through the digestive system before the larvae were sampled.

If a large proportion of a population is exposed to contaminants during early life stages, impacts on subsequent recruitment may be significant (Sindermann 1993). Estimates on how much of the 1989 Pacific herring year-class in PWS was affected by the oil spill vary from 4% (Pearson et al. 1995) to 50% (Brown et al. 1996). Pacific herring spawning events in

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PWS in 1989 were among the largest recorded, but through 1996, recruitment of the 1989 year-class into the fishery was among the lowest of any year-class since reliable estimates of year-class size were made in the early 1970s (J. Wilcock, Alaska Department of Fish and Game, Cordova, personal communication). Although larval lesions and mortality might have contributed to poor recruitment, recruitment of the 1989 year-class was also poor in Sitka Sound (G.D. Marty and Alaska Department of Fish and Game, unpublished data), which was used as a reference site for the PWS Pacific herring population (Pearson et al. 1995; Hose et al. 1996). Therefore, oceanographic variables probably were more significant in limiting recruitment of the 1989 year-class in PWS than was the oil spill.

In summary, Pacific herring larvae sampled from oiled sites after the 1989 *Exxon Valdez* oil spill had significant morphologic, histologic, and cytogenetic lesions, and the relation of these lesions to oil exposure was supported by laboratory study. Furthermore, histopathology and cytogenetic analysis were useful for detecting subtle lesions in wild fish larvae, and use of these techniques is recommended for damage assessment of future toxic spills. McGurk and Brown 1996) estimated that egg–larva mortality at the oiled sites was twice that of the two unoiled areas, and the high prevalence of ascites in larvae from oiled areas is consistent with those estimates.

Acknowledgments

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Performance of two red king crab pot designs

Shijie Zhou and Thomas C. Shirley

Abstract: We conducted a laboratory experiment to compare effectiveness of two pot designs in reducing the bycatch of female and sublegal-sized male red king crabs (*Paralithodes camtschaticus*) while retaining legal males. One pot (pot A) was a simulated standard king crab pot; a newly designed pot (pot B) had smaller mesh size, lower and wider entrances, and one-way opening triggers. Pot B prevented crabs from inserting their chelipeds through the mesh, motivated crabs to search for the entrance, eased the entry for all crabs, and facilitated the escape for all crabs but legal-sized males. Given that crabs had approached the pot, the mean entry probability for all crabs was 0.63 for pot B and only 0.13 for pot A. The probability of escape for females and sublegal males averaged 0.72 for pot B compared with 0.28 for pot A. As a result, the new pot design reduced the catch probability of females and sublegal males by over 60% while it increased the catch probability of legal males by over 25%.

Résumé : Nous avons réalisé une expérience de laboratoire pour comparer l'efficacité de deux casiers de conception différente pour réduire la prise accidentelle de crabes royaux (*Paralithodes camtschaticus*) femelles et de mâles de taille inférieure à la taille légale tout en conservant les mâles de taille légale. Le premier casier (casier A) est une réplique d'un casier standard utilisé pour la capture du crabe royal et l'autre (casier B) est un nouveau casier présentant des mailles plus petites, des entrées plus basses et plus larges et des dispositifs d'ouverture unidirectionnels. Le casier B empêchait les crabes d'insérer leurs pattes chéliformes à travers les mailles, motivait les crabes à rechercher l'entrée, facilitait l'entrée de tous les crabes et facilitait l'échappée de tous les crabes, sauf des mâles de taille légale. En supposant que les crabes se sont approchés du casier, la probabilité moyenne d'entrée pour tous les crabes était de 0,63 pour le casier B comparativement à seulement 0,13 pour le casier A. La probabilité d'échappée pour les femelles et les mâles de taille inférieure à la taille légale s'élevait en moyenne à 0,72 pour le casier B comparativement à 0,28 pour le casier A. En conséquence, la nouvelle conception du casier a réduit la probabilité de capture des femelles et des mâles de taille inférieure à la taille légale de plus de 60 %, tout en augmentant la probabilité de capture des mâles de taille légale de plus de 25 %.
[Traduit par la Rédaction]

Introduction

The red king crab (*Paralithodes camtschaticus*) fishery in Alaska is regulated by harvest rate and size, sex, and season (3-S management system) (Kruse 1993). A standard king crab pot used in the commercial fishery retains more than 60% of the bycatch of female and sublegal-sized male crabs (Zhou and Shirley 1996), which must be returned immediately to the sea. Ideally, fishing gear only catches legal male crabs. However, our previous study revealed that the king crab pots in current use do not provide effective size and sex selectivity (Zhou and Shirley 1997).

It is well known that catch rate and selectivity are affected by trap designs. In other trap fisheries, the number, size, and location of entrances have been shown to be important factors regulating the catch selectivity (Boutillier 1985; Christian et al. 1987; Kessler 1969; Miller 1980, 1990). In addition, one-way triggers in the entrances reduce escape from traps (High 1976; Hughes et al. 1970), and escape openings permit sublegal-sized individuals to escape (Brown 1982; Everson et al. 1992; Fogarty and Borden 1980; Nulk 1978; Paul 1981; Smith and Jamieson 1989; Weber and Briggs 1983). Altering the mesh size is a convenient method for regulating catch selectivity (Marshall and Mundy 1985; Miller 1990). The possibility exists for increasing the catch of legal male red king crabs while reducing that of sublegal males and female crabs by designing a better crab pot. However, little work has been conducted on selectivity of king crab pots.

We designed a new pot to increase the catch of legal male red king crabs while decreasing the catch of sublegal males and females based on our prior behavioral investigations (Zhou and Shirley 1997). The purpose of this paper is to demonstrate the improved catch selectivity of the new pot design compared with the pots used in the fishery. Experiments were conducted in a laboratory tank with crab behavior being observed and recorded by a closed-circuit video system and time-lapse video recorder. To evaluate pot performance, we emphasized the conditional probabilities of entry, escape, and catch.

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S. Zhou[1] and T.C. Shirley. Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska–Fairbanks, 11120 Glacier Highway, Juneau, Alaska 99801, U.S.A.

[1] Author to whom all correspondence should be addressed. Present address: Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, P.O. Box 25526, Juneau, AK 99802, U.S.A. e-mail: shijiez@fishgame.state.ak.us

Materials and methods

Crab collections

Red king crabs were collected with crab pots near Juneau, Alaska, and kept in laboratory tanks supplied with flowing seawater. Crabs were categorized into the following four groups: ovigerous females (OF), females without eggs (i.e., juvenile female, JF), legal-sized males (LM, ≥178 mm carapace width, CW), and sublegal-sized males (SM, <178 mm CW). All crabs were tagged on the merus of a walking leg with a piece of aluminum for identification in the video. Crabs

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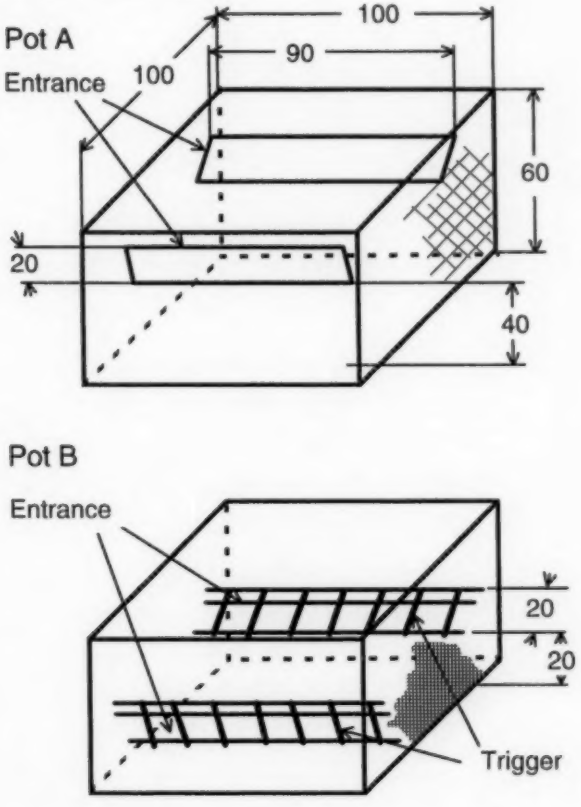
Fig. 1. Dimensions (cm) of simulated standard king crab pot (pot A). The newly designed pot (pot B) is the same size and shape as pot A but has two lower and wider entrances, one-way opening triggers, and smaller mesh.



were fed fish and squid twice a week but were deprived of food 2 days before experiments. Crabs with any indication of molting were not used. All crabs were measured for carapace length (CL, the straight line distance across the carapace from the posterior margin of the right eye orbit to the medial-posterior margin of the carapace) and width (CW). Sizes ranged from 64.9 to 181.9 mm CL (115.3 ± 22.4 mm: mean ± SD), and from 74.3 to 222.0 mm CW. We also measured the total body length (TL, the straight line distance from the tip of the rostrum to the posterior end of the abdomen), chela size, and body height (BH, the point where the distance between ventral and dorsal surfaces was greatest) to determine optimum parameters in pot design.

The king crab pot and the bait

Standard commercial king crab pots are square with length and width dimensions ranging from 150 to 240 cm and height from 67 to 99 cm. The pot has two tunnels at opposite ends, two side panels, one top panel and one bottom panel. The entrance frames at the end of the tunnel vary from 89 × 19 to 102 × 20 cm. Mesh sizes between 9 and 20 cm are used on various pots (unpublished data; High and Worlund 1979). A pot with dimensions of 100 × 100 × 60 cm was used as a simulated standard king crab pot in this study. The two entrance openings were 90 × 20 cm. Tar-treated knotted nylon mesh of 15.2 cm stretch mesh was used for the web. This simulated standard pot is referred to as pot A in this paper (Fig. 1).

The new pot (pot B) which we designed had the same dimension and shape as pot A, but the tunnels, entrances, and mesh size differed. The two entrances were lowered from 40 cm in pot A to 20 cm, and widened from 90 cm in pot A to the entire length of one side of the pot (100 cm; Fig. 1). Vertical bars (made from PVC pipe) with the upper end attaching to a horizon reebar and the down end free (called triggers) were rigged on each entrance to allow inward opening only. The gap between the two triggers measured 13 cm wide by 17 cm high. Mesh size was 4.7 cm.

Five hundred grams of frozen salmon cut in cubes was used in a 1-L cylindrical perforated jar (9 cm × 20 cm high) as bait for both pots. The jar was hung in the middle of the pot and 25 cm off the floor.

Experimental tank and observation system

We conducted our experiments in a round covered outdoor tank 5 m in diameter and 1.6 m in height. The tank was equipped with one horizontal inflow pipe and one standpipe for outflow. The pot was placed between the center and the wall of the tank with entrances parallel to the current direction. Filtered sea water from a depth of 30 m at ambient temperature and salinity flowed into this tank and filled it to a depth of 0.7 m. The inflow pipe paralleled the tank wall at 20 cm above the tank bottom and 20 cm from the tank wall. Water circulated in a clockwise direction with an exchange rate of 17.8 ± 0.3 $L \cdot min^{-1}$ ($n = 7$). At the tank center the current speed was close to zero, and it increased linearly toward the tank wall. The maximum speed was approximately 3.0 $m \cdot min^{-1}$ near the wall at 20 cm off the bottom. The water temperature ranged from 3.0 to 4.5°C, while the salinity remained near 30 ppt, during the experiments.

Four fluorescent lights of 33 W each and a standard light of 100 W provided 8–11 lx illuminance at the water surface. Two low-light video cameras (Cohu solid state camera, Model 4815-5000, and FOCUS Vision 4, Model FS-412) were connected to a time-lapse VCR (GYYR TLC 1800) and two monitors in an observation room. Crab behavior was recorded continuously on video tape and then analyzed later.

Experimental protocol

After being deprived of food for 2 days, 16 crabs, 4 each of categories OF, JF, LM, and SM, were transferred into the experimental tank. After 20 h of acclimatization to the tank, one pot (either pot A or B, depending on the sequence) was placed in the tank, then bait was placed in the center of the pot 25 cm from the bottom. The behavior of all crabs was observed and recorded for 48 h. At the end of each trial, crabs inside the pot, if any, were released into the tank and the pot removed. Then the other pot was placed in the same position, the water was mostly, but incompletely, drained and replaced, but the crabs were not replaced. After a 1-day interval, the trial continued with the same crabs for another 48 h. Crabs were replaced after being tested with both pot A and pot B. The test order of the two pots was alternated in different trials to minimize the effect of test order. A total of 144 crabs was used in 9 trials.

Some activities related to the pot were defined as follows.

(1) Foraging occurred when a crab moved about on the tank bottom and appeared to be searching for food.

(2) Approach occurred when a crab walked toward the pot and contacted the pot with its anterior body. While touching the pot, the crab moved actively in a searching mode for food. This definition excluded the specific behavior of contacting the pot while moving backward or laterally without probing the pot mesh and also excluded crabs that touched the pot lightly with the front of their carapace but were not moving actively.

(3) Entrance search occurred from when a crab approached the pot to when it either entered (see "entry" below) the pot or left (see "leaving" below) the pot. During an entrance search, crabs typically made short excursions around the pot and appeared to find the entrance, or inserted their chelipeds through the mesh, by which they appeared to attempt to force their way into the pot.

(4) Leaving occurred when the crab, after entrance searching, moved

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away from the pot by at least one body length or width (including the appendages) and for at least 1 min. If the crab returned and touched the pot again from a distance of less than one body length or width and within 1 min, then the behavior was not scored as leaving, but rather as an approach.

(5) Entry occurred when a crab entered the pot and released its hold on the entrance.

(6) Escape occurred when a crab inside the pot left through the entrance and broke contact with the entrance.

Data analysis

Data were statistically analyzed to detect differences in crab responses between the two pot designs and among the four crab categories. Before and after statistical analysis, data were graphically examined for outliers, collinearity, independence, normality, and homogeneity of variance. Parametric statistical methods (e.g., ANOVA) were our first choice if data did not violate the assumptions. Data transformation was applied when necessary. If parametric methods were not appropriate, then nonparametric methods (e.g., Kruskal–Wallis test and G test) were used.

Results

Pot design and crab morphology

Morphological differences of red king crabs provide a means by which pot design can select for size. After maturity the growth rate of females is slower than that of males, and most female crabs do not grow larger than the minimal size for legal crabs (Marukawa 1933; Bright 1967). Therefore, most females caught in the fishery are smaller than legal-sized males (Zhou and Shirley 1996). We observed that TL and BH were critical dimensions in entering and escaping from the pot and estimated the relationship between these two dimensions and CW (Fig. 2)

For male crabs, when the CW of 178 mm was set as the minimal legal size, TL equaled 182.8 mm, and BH was approximately 94.3 mm. To retain legal males while allowing sublegal males and most females to escape, the diagonal distance of the opening that is formed by two adjacent triggers and the upper and lower shafts of the entrance should be at least equal to a TL of a minimum legal-sized male crab. We chose 170 mm as the gap between two triggers and 130 mm as the height of the entrance for the new pot design. These dimensions provide an opening with a diagonal distance of 214 mm.

We also observed that mesh size appeared to affect crab behavior while the crab was searching for the entrance. A mesh size small enough to prevent crabs from inserting their chelae into the pot appeared to motivate an entrance search. The circumference of chela (S) was measured, and its relationship with CW was

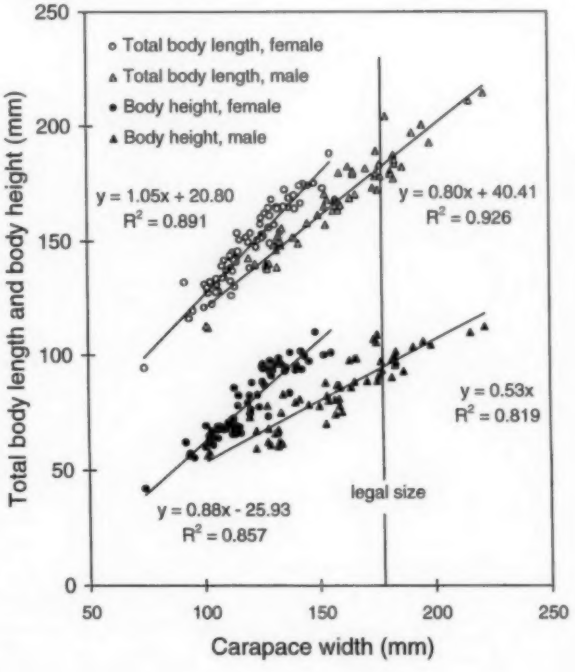
$$\text{small (left) chela: } S_s = 0.46CW - 1.8,\ R^2 = 0.920,\ n = 133$$

and

$$\text{large (right) chela: } S_l = 0.89CW - 18.2,\ R^2 = 0.896,\ n = 134$$

The legal males (≥178 mm CW) had a minimum S_l of 140 mm while the minimum S_s was 80 mm. To prevent legal males from inserting their chelipeds into the pot, we used a mesh size of 47 mm (94 mm in circumference) for pot B.

Fig. 2. The relationship between total body length and carapace width, and between body height and carapace width for male (n = 60) and female (n = 76) red king crabs. The minimal legal size is 178 cm CW.



Entrance search

When a crab approached the pot, it actively moved about to search for the entrance. This entrance searching behavior differed between pot A and B. While approaching pot A, crabs typically inserted their chelipeds through the mesh and forced their bodies against the panel. After trying ineffectively to enter the pot for a while, crabs often withdrew their appendages, shifted to a new location, and poked their chelipeds into the mesh again. When approaching pot B, most crabs touched and grasped the mesh, but could not insert their chelipeds through it because the mesh size was too small. Therefore, at pot B crabs moved more frequently and remained a shorter time at one location than at pot A.

During each entrance search, crabs moved around the pot periphery. The range of movement subtended an angle at the center of the pot, defined as search angle. The frequency distribution of the search angles of individual crabs differed significantly between pots A and B (Kolmogorov–Smirnov test, $p < 0.05$), with more crabs searching a narrower angle around pot A than pot B. However, when analyzed by ANOVA, the mean search angle did not differ between the two pots (71.6 ± 36.0° (mean ± SE), n = 320 around pot A and 73.8 ± 34.7°, n = 109 around pot B, $p > 0.05$).

The duration of each entrance search ranged from less than 1 to 109 min with a mean of 2.9 ± 9.2 min (mean ± SD) for pot A and from less than 1 min to only 20 min with a mean of 1.7 ± 2.9 min for pot B. Duration of search differed significantly between the two pots after logarithmic data transformation (ANOVA, $p < 0.001$).

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Fig. 3. Conditional probability of entry (P(I|A)) given that an approach occurred for pot A and pot B. JF, Juvenile females; OF, ovigerous females; SM, sublegal males; LM, legal males.

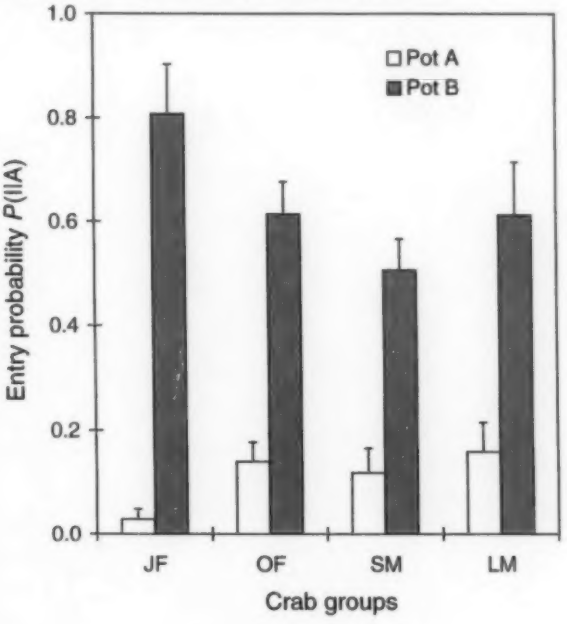
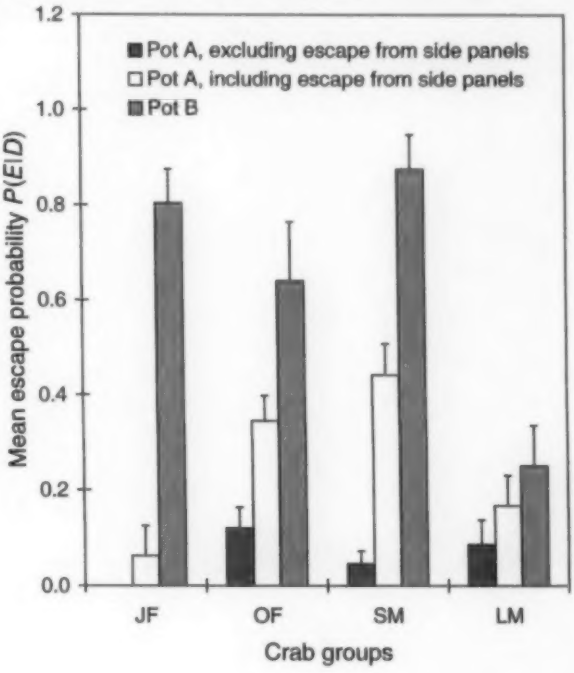


Fig. 4. Mean escape probability (no. of crabs escaped/maximum no. of crabs in the pot). Pot A included or excluded escapes starting from side panels. All juvenile females escaped by starting from side panels. Pot B included escapes starting from all locations.



Entry probability

Behavioral differences were observed when crabs entered the two pots. After approaching pot B, a crab often crawled straight up the tunnel, pushed the triggers open, and entered the pot in forward position in a short time. In contrast, after approaching pot A, a crab frequently moved back and forth and turned its body around in the tunnel. When the entrance was located, the crab might not face forward, which resulted in a higher rate of entry in directions other than forward in pot A than in pot B.

The conditional probability of entry given that crabs approached (P(I|A), where I is ingress and A is approach) was significantly affected by pot design (ANOVA, $p < 0.001$). Pot B had a higher P(I|A) for all crabs than pot A. The analysis failed to find a significant difference among crab groups, but pot types and crab groups showed an interaction ($p = 0.026$): JF had the lowest P(I|A) in pot A but highest in pot B (Fig. 3). On average, the entry probability of females and sublegal males increased from 0.096 ± 0.096 (mean ± SE) in pot A to 0.643 ± 0.073 in pot B and that of legal males also increased from 0.159 ± 0.056 in pot A to 0.612 ± 0.103 in pot B, an increase of 285%.

Escape probability

Most crabs (71%) escaped from pot A through the two side panels, which was assumed to be difficult for standard commercial pots. When crabs started an escape from the side panel, the legs on one side of the body held onto the side panel while legs of the other side extended to the entrance. After the legs on the other side touched the entrance, they contracted and pulled the crab over to escape. Because the distance between the side panel and the entrance was only 10 cm in pot A, crabs could easily escape in this way. However, this distance is much greater in standard king crab pots (approximately 50 cm in most pots), and crabs have difficulty escaping by starting from a side panel. For example, Stevens et al. (1993) observed that of 20 escapes from standard king crab pots, only 3 started from side panels. Therefore, we presented the escape data both with and without escapes from the side panels for pot A. The escape probability was calculated in 12-h intervals and expressed as the number of crabs escaped (E), given the maximun number (D) of that group of crabs in the pot during that period: $P(E|D) = E/(E+R)$, where R was the number of that group of crab retained at the end of the period.

When escapes from side panels were included, SM had a significantly higher $P(E|D)$ than JF (0.44 vs. 0.06, Fisher's exact test, $p = 0.019$), but no significant difference was found between any other crab groups (G test, $p \geq 0.062$). When escapes using side panels were excluded, the $P(E|D)$ was low for all crab groups (mean $P(E|D) \leq 0.10$), and no significant difference was detected between groups (Fig. 4).

When escaping from pot B, all crabs exited laterally through the gap between two triggers. Because of size, legal males (LM) had a significantly lower $P(E|D)$ than other crabs, (G test, $p < 0.001$), but no significant difference existed among JF, OF, and SM (G test, $p = 0.480$). The mean $P(E|D)$ were 0.80, 0.64, 0.88, and 0.25 for JF, OF, SM, and LM, respectively (Fig. 4). On average, females and sublegal males had a 0.52 higher $P(E|D)$ than legal males.

Escape probabilities significantly differed between pot A and pot B (G test, $p \leq 0.001$). The mean $P(E|D)$ for OF, JF,

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Fig. 5. The relationship between the number of crabs retained in the pot and the soak time for pot A and pot B. Bait was put in the pot at time 0. Escapes from side panels were excluded for pot A.

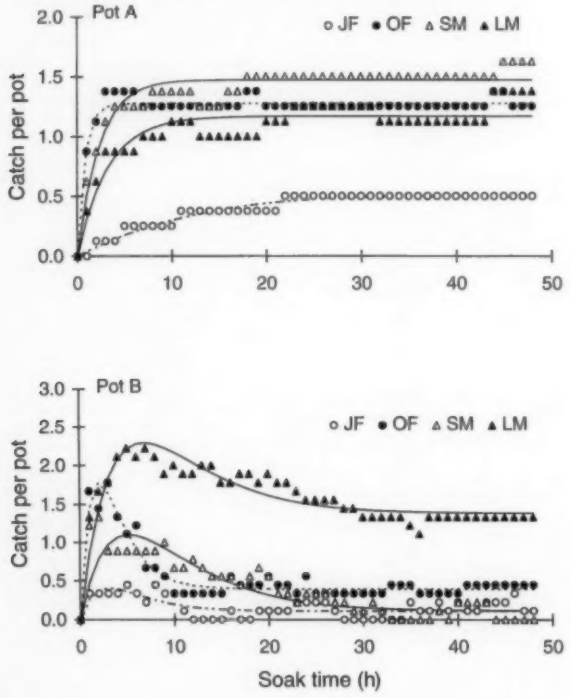
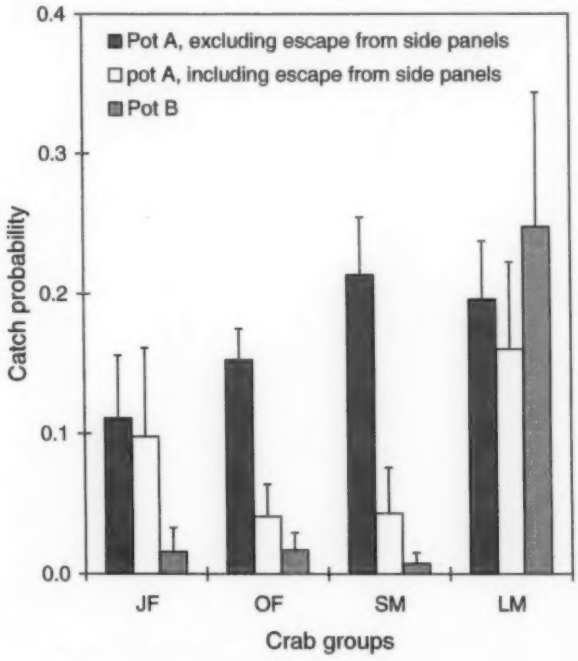


Fig. 6. Catch probability given that an approach occurred for pot A and pot B. Pot A included or excluded escapes starting from side panels.



and SM from pot B was 0.44 and 0.67 higher than that from pot A, including and excluding escapes using side panels, respectively. However, the $P(E|D)$ of LM was similar between the two pots (G test, $p = 0.339$). Clearly, the lower entrances and triggers in pot B selectively retained legal-sized males while increasing the escape of females and sublegal males.

Duration of confinement inside the pot also indicated the difficulty of escape. For crabs that escaped, this duration ranged from 0.4 to 38.2 h for pot A (including escape via side panels) and from 0.1 to 35.5 h for pot B. Legal males were confined for the shortest mean time (7.8 ± 9.7 h; mean ± SD) in pot A, but longest (12.2 ± 10.5 h) in pot B. On average, OF, JF, and SM were trapped inside pot A for 13.7 ± 11.5 h but for only 3.2 ± 4.9 h in pot B (ANOVA, $p < 0.001$).

Catchability

The dynamics of entry and escape resulted in a change in the number of crabs retained in the pot over time (Fig. 5). Because of low escapes, Pot A tended to have an asymptotic catch for all crabs over time, while the number of female and sublegal male crabs decreased quickly after several hours in pot B.

Pot performance can be evaluated overall by catch probability given crabs approached the pot. Both pot type and crab categories significantly affected the catch probability (ANOVA, $p = 0.031$ for pot type when escape from the side panels was included; $p = 0.001$ for crab group). When escape from the side panels was excluded from pot A, the differences were more marked ($p < 0.001$ for pot types; $p = 0.006$ for crab group). Also pot type and crab groups interacted significantly ($p = 0.018$). On average, pot B reduced the catch probability of females and sublegal males by 62.9% (when pot A included escape from side panels) or 92.2% (when pot A excluded escapes from side panels). At the same time, pot B increased the catch probability of legal males by 55.0% (when pot A included escape from side panels) or 26.5% (when escape from side panels was excluded) (Fig. 6).

Discussion

Mesh size appeared to affect entrance search behavior of crabs. A mesh size smaller than chela size may motivate crabs to search around the pot and thereby increase the probability of finding an entrance. However, although crabs did move more frequently while contacting pot B, they were apt to make excursions left and right within a narrow range downstream of the bait. Because decapods are strongly attracted by bait odor while searching (Miller 1980, 1990), the strategy of forcing crabs to search by using smaller mesh size requires further exploration.

Access into the pot is one of the most important factors affecting the catch. Ideally, the entry probability given that approach occurred [$P(I|A)$] should equal one for legal crabs but zero for sublegals and females. Our studies indicated that, since all crabs behaved similarly in searching and entering the pot, selectively permitting the entry of legal males appears to be difficult. Although the pot with higher entrances prevented more small crabs from entering than the pot with lower entrances, it also greatly reduced the entry of legal crabs. As a compromise, we chose to improve entry rates for all crabs as

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a strategy in the new pot design. Wide and low entrances proved to be efficient in pot B.

The incorporation of escape openings in traps has been used widely in decapod fisheries as a technique to reduce the catch of sublegal-sized animals (Brown 1982; Everson et al. 1992; Fogarty and Borden 1980; Krouse 1978; Weber and Briggs 1983). Our observations and those of others (Brown 1982) showed that escape depends upon total body length and height rather than carapace width. Because decapods possess a rigid exoskeleton and are able to orient themselves to an optimum position for escape, the catch selectivity can be precise when rigid material is used for the openings (Miller 1990).

Our new design included low tunnels and precisely determined gaps between triggers as means of escape for sublegals rather than escape vents. Clearly, pot B functioned well for this task. If we averaged the probability of escape for all females and sublegal males, $P(E|D)$ for pot B was 0.72, while for pot A it was 0.28 when escapes from side panels were included, and only 0.06 when escapes from side panels were excluded. For legal males, $P(E|D)$ of 0.25 for pot B seemed higher than pot A of 0.17. The small size of legal crabs used may have contributed to this result, since several "legal males" used in the study were near the minimum legal size (178 mm CW). Also, the dimensions of entrance openings between triggers (170 × 130 mm) may have been too large to retain all legal crabs.

The high probability of escape from pot B may not only reduce the retention of female and sublegal male crabs but also promote the catch of legal males. Gear saturation and agonistic interactions between individuals inside and outside traps are factors that affect the catch (Miller 1979, 1990; Bennett 1974; Castro and Dealteris 1990). Traps with escape vents reduce the number of sublegal lobsters and crabs while increasing the catch of legal ones (Fogarty and Borden 1980; Brown 1982; Krouse 1978; Smith and Jamieson 1989; Weber and Briggs 1983). The retention of female and sublegal male crabs has been estimated as high as 64.6% in Bristol Bay red king crab fishery during 1991–1993 (Zhou and Shirley 1996). It is possible to reduce this bycatch and increase the catch of legal crabs by employing new gear, such as pot B.

Different conditions in the laboratory study and commercial fishery must be kept in mind when applying our results to the field. Field conditions include high crab density, large-sized pots, variable pot soak times, large quantities of chopped bait combined with an entire Pacific cod *Gadus macrocephalus* (as hanging bait), and changes in current. These are difficult to simulate in the laboratory. Such differences may diminish the effectiveness of the new design. A large-scale field test is warranted.

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Phylogenetic analysis of Pacific salmon (genus *Oncorhynchus*) using nuclear and mitochondrial DNA sequences

M.J. Domanico, R.B. Phillips, and T.H. Oakley

Abstract: Recent phylogenetic analyses of Pacific salmon of genus *Oncorhynchus* based on sequences from mitochondrial DNA and one nuclear growth hormone intron supported two subgroups:one with chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon and the other with pink (*O. gorbuscha*), sockeye (*O. nerka*), and chum (*O. keta*) salmon. In the latter group, a sister relationship was indicated between pink and chum salmon. Previous studies based on morphological and allozyme data had suggested a closer relationship between pink and sockeye salmon. In this paper we present a combined analysis of 4365 base pairs aligned sequence from nuclear and mitochondrial genes including new sequence data from the internal transcribed spacers (*ITS1* and *ITS2*) of the nuclear ribosomal DNA, the sequences of three growth hormone introns (*GH1C*, *GH2C*, *GH2D*) and the sequences of the *ATPase6* and *ND3* genes of the mtDNA. Phylogenetic analysis of the combined data set gives strong support to a close relationship between pink and chum salmon and between coho and chinook salmon.

Résumé : Des analyses phylogénétiques récentes du saumon du Pacifique du genre *Oncorhynchus* fondées sur des séquences d'ADN mitochondrial et d'un intron d'une hormone de croissance nucléaire ont révélé l'existence de deux sous-groupes : un qui comprend le saumon quinnat (*O. tshawytscha*) et le saumon coho (*O. kisutch*) et l'autre comprenant le saumon rose (*O. gorbuscha*), le saumon sockeye (*O. nerka*) et le saumon kéta (*O. keta*). Dans ce dernier groupe, une relation soeur a été observée entre le saumon rose et le saumon kéta. Des études antérieures fondées sur des données morphologiques et des données sur les allozymes avaient laissé entendre une relation plus étroite entre le saumon rose et le saumon sockeye. Dans le présent article, nous présentons une analyse combinée d'une séquence de 4365 paires de bases alignées provenant de gènes nucléaires et mitochondriaux, y compris des données sur la nouvelle séquence provenant des espaceurs transcrits internes (*ITS1* et *ITS2*) de l'ADN ribosomique nucléaire, les séquences de trois introns d'hormone de croissance (*GH1C*, *GH2C*, *GH2D*) et les séquences des gènes *ATPase6* et *ND3* de l'ADNmt. L'analyse phylogénétique de l'ensemble de données combinées vient appuyer fortement l'existence d'une relation étroite entre le saumon rose et le saumon kéta et entre le saumon coho et le saumon quinnat.
[Traduit par la Rédaction]

Introduction

The salmonid genus *Oncorhynchus* includes chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), Asian masu salmon (*O. masou*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), sockeye salmon (*O. nerka*), and trouts native to the North Pacific that were formerly included in the genus *Salmo* (Smith and Stearley 1989). Evolutionary relationships among *Oncorhynchus* species have been studied by comparing morphologies (Stearley 1992), allozymes (Utter et al. 1973), mitochondrial DNA (mtDNA) (Thomas et al. 1986;Thomas and Beckenbach 1989; Shedlock et al. 1992; Domanico and Phillips 1995), short interspersed repetitive elements (SINES) (Murata et al. 1993, 1996), ribosomal DNA (rDNA) restriction fragment length polymorphisms (RFLPs) (Phillips et al.1992), the nucleotide sequence of the D intron of growth hormone 2 (*GH2D*) (McKay et al. 1996) and mitochondrial DNA (mtDNA) sequence data (Domanico and Phillips 1995). Most of these studies found Asian masu salmon to be intermediate between the rainbow trout and other Pacific salmon species. Among Pacific salmon, two subgroups usually contained coho and chinook salmon, and pink, sockeye, and chum salmon, respectively.

Different data sets have supported different relationships among pink, sockeye, and chum salmon (reviewed in Smith 1992). Allozymes (Utter et al. 1973), rDNA RFLPs (Phillips et al. 1992), mtDNA D-loop sequences (Shedlock et al. 1992), and morphology (Stearley 1992) suggest that pink and sockeye are sister species. Life histories (Smith and Stearley 1989), mtDNA RFLPs (Thomas et al. 1986), mtDNA sequence data from two protein-coding genes (*ND3* and *ATPase6*) (Thomas and Beckenbach 1989; Domanico and Phillips 1995), nuclear repetitive elements or SINEs (Murata et al. 1993), and nuclear sequence data from the *GH2D* intron (McKay et al. 1996) suggest an alternative relationship in which pink and chum are sister species.

To resolve conflicting trees for Pacific salmon and to compare relationships suggested by nuclear DNA data with those indicated by mtDNA, we sequenced the internal transcribed spacers (*ITS1* and *ITS2*) of the nuclear rDNA. The *ITS1* and *ITS2* were chosen for this analysis because they are evolving rapidly compared with other nuclear regions (reviewed in Gerbi 1985). The spacers are located within the rDNA repeat

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M.J. Domanico, R.B. Phillips,[1] and T.H. Oakley. Department of Biological Sciences, University of Wisconsin, Milwaukee, WI 53201, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: rp@csd.uwm.edu

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Fig. 1. Aligned DNA sequence of the first internal transcribed spacer (*ITS1*) of ribosomal DNA for seven *Oncorhynchus* species. Sites in common with the first sequence are indicated by periods, gaps are indicated by minus signs, and phylogenetically informative sites are indicated by plus signs. (Informative sites in bases 547–586 were ignored in the analysis because alignment of this region was problematic.)

with *ITS1* between the 18S and 5.8S and *ITS2* between 5.8S and 28S RNA genes. Phylogenetic analysis of *ITS* sequences has clarified phylogenies of a number of different species including fishes of the genus *Salvelinus* (Pleyte et al. 1992; Phillips et al. 1994), primates (Gonzales et al. 1990), and several invertebrates (Vogler and DeSalle 1994; Fritz et al. 1994).

We have obtained additional nuclear sequence data from two growth hormone introns, *GH1C* (Oakley 1996) and *GH2C* (Domanico 1994) and combined these with sequences of the *GH2D* intron (McKay et al. 1996). In this paper we present results of a combined phylogenetic analysis of all of the DNA sequence data currently available (4365 bp) from Pacific salmon using rainbow trout as an outgroup.

Materials and methods

Materials and extractions

Fish samples used for determination of ITS sequences were obtained from the following sources: rainbow trout (Evergreen Hatchery, Pound, Wisconsin) chinook salmon (Warm Springs Hatchery, Washington), coho salmon (Juneau, Alaska), Asian masu salmon (Japan), chum salmon (Juneau, Alaska), sockeye salmon (Bristol Bay, Alaska), and pink salmon (Juneau and Prince William Sound, Alaska). DNA was extracted from the liver tissue of two fish from each sampling area, except for masu salmon where only one fish was used. Either the Stratagene kit or phenol was used to extract DNA (Sambrook et al. 1989).

Amplification and sequencing of the rDNA spacers

The region of rDNA spanning the *ITS1* and *ITS2* and including the 5.8S gene were amplified together as one fragment using the polymerase chain reaction (PCR) (Saiki et al. 1988). The 18S rDNA sequence from *Xenopus laevis* (Salim and Maden 1981) was used to design the forward primer (5′ CTTGACTATCTAGAGGAAGT 3′) and the 28S sequence from *Xenopus laevis* (Ware et al. 1983) was used to design the reverse primer (5′ ATATGCTTAAATTCAGCGGG 3′).

Amplifications of rDNA were carried out as described in Phillips et al. (1995*b*) using a 5′ biotinylated reverse 28S primer to facilitate production of single stranded DNA. Dynabeads M-280 streptavidin (Dynal, Inc.) were used according to the manufacturer's instructions for separating double-stranded PCR product into single-stranded DNA for use in the sequencing reactions.

Dideoxy sequencing reactions (Sanger et al. 1977) were done using Sequenase version 2.0 (U.S. Biochemical) according to the manufacturer's instructions. Sequencing primers used for sequencing the *ITS1* region were KP2 and 5.8S (Phillips et al. 1995*b*). The KP2 primer was used to sequence the 5′ portion (18S side) of the *ITS1* and the 5.8S primer was used to sequence the complimentary strand to obtain the sequence of the 3′ portion of the spacer. Nucleotide sequences of both strands were determined with an overlapping region of approximately 100 base pairs. The sequencing primer for the *ITS2* was 5′ CTACGCCTGTCTGAGTGTC 3′, which was designed from the 5.8S gene of rainbow trout (Nazar and Roy 1978) and was used to sequence the entire *ITS2*. Sequences were aligned using CLUSTALV (Higgins et al. 1992) and adjusted by eye. Percent sequence divergences were determined by calculating the absolute genetic distances with the PAUP program (Swofford 1993) and genetic distances calculated using the Jukes–Cantor method with the MEGA program (Kumar et al. 1993).

Phylogenetic analysis of rDNA sequences

The most parsimonious trees were determined using the exhaustive search option and majority rule consensus trees were constructed using the branch and bound method of PAUP (Swofford 1993) with 500 bootstrapped replications. Gaps were analyzed in two ways: (*i*) by treating gaps as missing data and (*ii*) by treating gaps as missing data and adding a separate binary character matrix based on presence or absence of each indel. Rainbow trout and Asian masu salmon were used as outgroups. For the final analyses a region of uncertain homology between bp 547 and 586 in the *ITS1* was excluded. This did not affect tree topology but increased character fitness statistics.

Combined analysis of nuclear and mitochondrial DNA sequences

For the combined analysis of all DNA sequences, gaps were treated as missing data. The most parsimonious (MP) tree was calculated using the exhaustive search method of PAUP. A majority rule consensus tree was also produced using the branch and bound method of PAUP with 500 bootstrapped replications. Jukes–Cantor distances were calculated and a phenogram constructed using the neighbor joining aligorithm of MEGA (Kumar et al. 1993) with 500 bootstrapped replications.

Results

Analysis of *ITS1* and *ITS2* sequences

Nucleotide sequences of the rDNA spacers (*ITS1* and *ITS2*) from seven *Oncorhynchus* species gave a final data set of 971 aligned base pairs, with *ITS1* containing 593 bp (Fig. 1) and *ITS2* containing 378 bp (Fig. 2).

When gaps were counted as missing data, there were 99 variable sites with 37 being phylogenetically informative. Percent sequence divergences ranged from 1.62 between pink and chum salmon to 8.29 between pink and chinook salmon (Table 1). The transition/transversion ratio averaged 1.06 (Table 2). Trees were rooted assuming masu salmon and rainbow trout as outgroups. One best cladogram of 49 steps was produced using PAUP (Fig. 3) with uninformative characters excluded. Chinook and coho cluster as sister species. Pink, sockeye, and chum form a clade and pink and chum show a sister relationship. A second best cladogram at 50 steps had the same topology except that chinook and coho were on separate adjacent branches

There were 79 indel characters (assuming contiguous gaps as single characters). Thirty five of the indel characters were phylogenetically informative. Combining these indel characters with the substitution matrix produced a single most parsimonious tree consistent with Fig. 3 only with higher bootstrap values.

A phylogenetic tree was also produced using the neighbor joining method of the MEGA program (Kumar et al.1993) with Jukes–Cantor distances (Table 3). This tree showed the same topology as those produced using the parsimony method of PAUP (Swofford 1993).

Combined nuclear and mitochondrial DNA sequence analysis

Data from three growth hormone introns (Devlin 1993; Du et al. 1993; Forbes et al.1994; Domanico, 1994; McKay et al.

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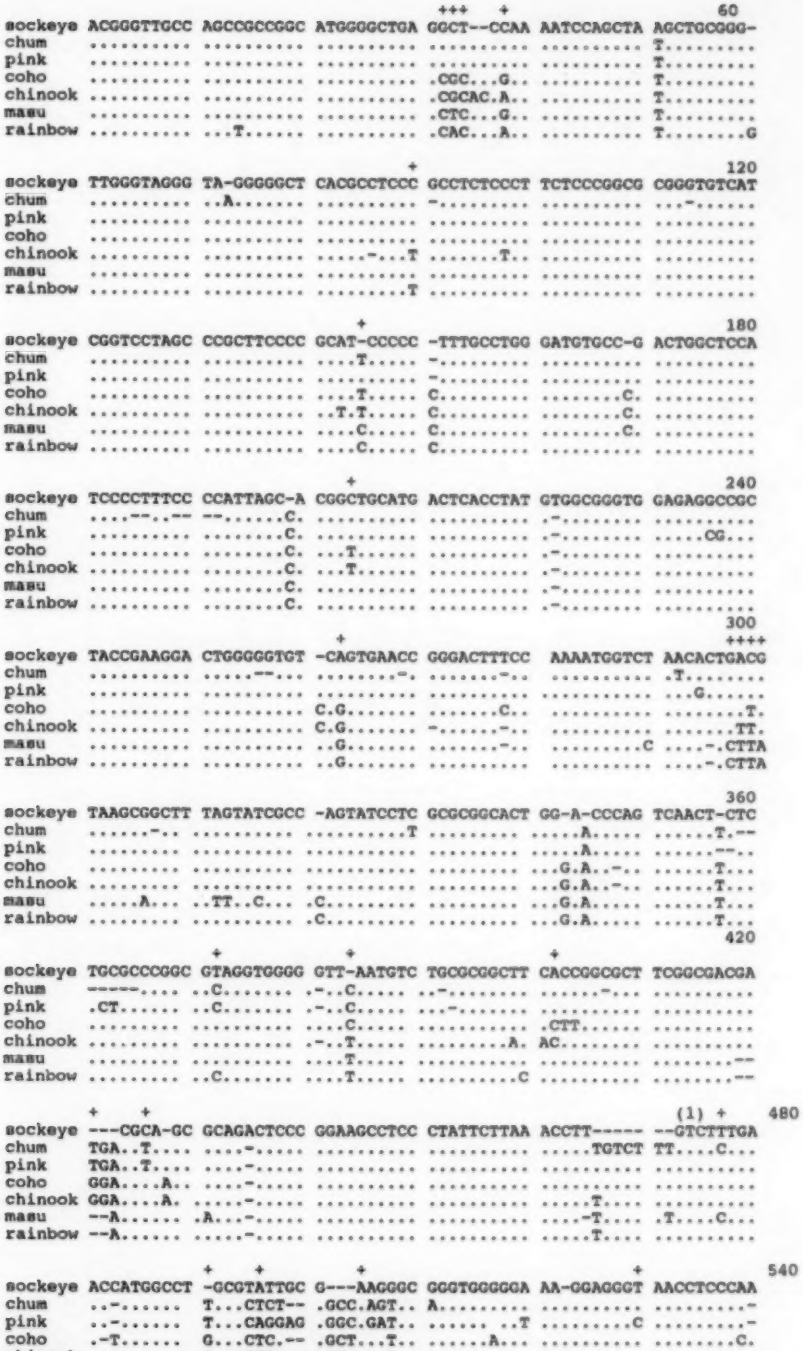

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Fig. 2. Aligned DNA sequence of the second internal transcribed spacer (*ITS2*) of ribosomal DNA for seven *Oncorhynchus* species. Sites in common with the first sequence are indicated by periods, gaps are indicated by minus signs, and phylogenetically informative sites are indicated by plus signs.

```
                                                                                                                  60
sockeye TGTCATCAAT CGGAACCTCT -GGTTTCCGC AGCTGGGGCA GT-GCAGGCG GCCACCGTGC
chum    .......... .......... G......... .......... ..C....... ..........
pink    .......... .......... G......... .......... ..C....... ..........
coho    .......... .......... G......... .......... ..C....... ..........
chinook .......... .......... G......... ..A....... ..C....... ..........
masu    .......... .......... .......... .......... ..C....... ..........
rainbow .......... .......... .......... .......... ..C....... ..........

                                                                    +++           120
sockeye AGCCTTCGTC CCCCTAAGTT CAGACCAGGA CGGCTCGGTG GGGTT-GTTG AGGATGAGCT
chum    .......... .......... T......... .......... ..T....... ..........
pink    .......... .......... A......... .......... ..T....... ..........
coho    .......... .......... .......... .......... ..TAGC.... ..........
chinook .......... .......... .......... .......... .TTAGC.... ..........
masu    .......... ...-...... .......... .......... ..T....... ....G.....
rainbow .......... ...-...... .......... .......... ..A.G..... ..........

                                                                          +    180
sockeye TCTG-CTCTA CTTCCG-A-T CCCCCGTGCG CTCTTCCTTT CCCTT-TCGC TTCG-CGAGA
chum    .......... .....GG..T .......... .......... .......... ....-.....
pink    .......... ......-..T .......... .......... .....C.... .C..-.....
coho    ....G..... .C....CT.T .......... .......... .....C.... .C..-.....
chinook .-..G..... .C....C-.T .......... .......... .....C.... .-..G.....
masu    .-..G....  ..-...C-.T .......... .......... .....C.... ....-.....
rainbow .-..G..... .C........ .......... .......... .....C.... ....G.....

                                                                          +    240
sockeye GGCGCCCACG TTCCCCGCAT GGTCTGGCGC GGCTGCCGGT GGACTCT-GT CTGTCCGTGC
chum    .......... .......... .......... .......... .......-.. ..C.......
pink    .......... .......... .......... .......... .......-.. ..C.......
coho    .......... .......... .......... .......... .....T.T.. ..C.......
chinook .......... .......... .......... .........- -AG....T.. ..T.......
masu    .......... .......... .......... .......... .......T.. ..T.......
rainbow .......... .......... .......... .......... ....A..T.. ..T.......

              +          +                                    +              300
sockeye TGCCCGTGTT ACGCATGTGG TTCTCGGGGT AGCGCTCGGG GTTAGGTTAG GGCTGCGGAG
chum    .......... .......C.. .......... .......... ........T. ..........
pink    .......... .......C.. .......... .......... ........T. ..........
coho    .........A .......... .......... .......... .......... ..........
chinook .-........A .........- .......... .......... .......... ..........
masu    .........A .......... .......... .......... .......... ...C......
rainbow .........A C......... .......... .......... .......... ...G......

                                                                +             360
sockeye CTCCGACCGC CGACCTGAAA CGTAATTGAG AAGGTGAGCC GGGCAACCGG CCACAATCCA
chum    .......... .......... ...-...... .......... .......... ..........
pink    .......... .......... ...-...... .......... .......... ..........
coho    ........A. .......... ...-...... .......... .......... ..........
chinook .....G-... ..T....... .......... .......... .......... ..........
masu    .......... .......... ...-...... .......... ....G..... ..........
rainbow .......... .......... .......... .......... ....G..... ..........

                          378
sockeye TTCACTTTGA CTACGACC
chum    .......... ........
pink    .......... ........
coho    .......... ........
chinook .......... ........
masu    .......... ........
rainbow .......... ........
```

1995; Oakley 1996) are available for all North American Pacific salmon. Growth hormone intron sequences (*GH1C*, *GH2C*, *GH2D*), rDNA sequences (*ITS1* and *ITS2*), and mitochondrial protein coding sequences (*ATPase6* and *ND3*) were combined into one data set, which contained 4365 aligned base pairs. Sequence alignments will be presented elsewhere and are available from the authors. Aligned sequences were analyzed using maximum parsimony (PAUP; Swofford 1993) with the indels considered missing data. The most parsimonius tree was consistent with the most parsimonius trees obtained with the rDNA spacers and mtDNA data. Bootstrap values were higher for the combined analysis (Fig. 4). This tree, based on combined data, had 234 steps (ignoring uninformative characters), with the next most parsimonious tree having 251 steps. There were a total of 143 informative sites (Table 4), with 35 synapomorphies between pink and chum salmon; 43 between pink, chum, and sockeye; and 26 between chinook and coho salmon.

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Table 1. Absolute distance of the *ITS1* and *ITS2* between seven *Oncorhynchus* species generated using PAUP.

	Sockeye	Chum	Pink	Coho	Chinook	Masu	Rainbow
Sockeye	—	0.0405	0.0324	0.0649	0.0600	0.0541	0.0514
Chum	15	—	0.0162	0.0610	0.0795	0.0725	0.0750
Pink	11	6	—	0.0649	0.0829	0.0750	0.0778
Coho	24	25	24	—	0.0231	0.0625	0.0575
Chinook	21	31	29	9	—	0.0500	0.0342
Masu	20	29	27	25	19	—	0.0300
Rainbow	19	30	28	23	13	12	—

Note: All alignment gaps were treated as missing data. Number of differences are given below the diagonal. Percent sequence divergences are given above diagonal.

Table 2. Transition to transversion ratios for the *ITS1* and *ITS2* DNA sequences for seven *Oncorhynchus* species.

	Sockeye	Chum	Pink	Coho	Chinook	Masu	Rainbow
Sockeye	—	1.3912	0.5847	0.9450	0.7060	1.2224	0.8326
Chum	0.6519	—	0.7531	1.5748	1.454	2.0519	1.1528
Pink	0.2824	0.4103	—	0.9547	0.7824	1.1391	0.6718
Coho	0.3569	0.6166	0.3387	—	1.3503	1.5300	1.3562
Chinook	0.2516	0.4004	0.2589	0.6011	—	1.2224	0.8073
Masu	0.4343	0.7668	0.3873	0.5776	0.4343	—	1.2147
Rainbow	0.3062	0.4150	0.2374	0.5246	0.3178	0.5256	—

Note: Transition/transversion ratios are given above the diagonal, and standard errors are given below the diagonal.

Fig. 3. Majority rule consensus tree based on 500 branch and bound bootstrapped replications of *ITS1* and *ITS2* DNA sequence data (971 bp). All gaps were counted as missing data. Informative sites in the section from bp 547 to 586 were ignored in the analysis, since this section had multiple plausible alignments. Numbers indicate the percentage that each branch was retained in 500 samplings of the data (parsimony bootstrap percentages above each node, Jukes–Cantor/neighbor joining percentages below each node). Rainbow trout and masu salmon were used as outgroups and the tree was rooted with a basal polytomy.

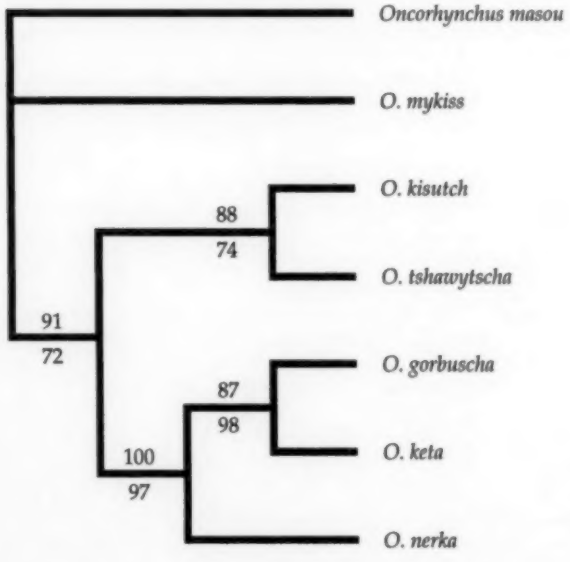

Fig. 4. Bootstrapped parsimony tree of the six *Oncorhynchus* species that represent the Pacific salmon using combined data set (4365 bp) of the sequences of the nuclear ITS1 and ITS2 rDNA, GH1C, GH2C, and GH2D and the mitochondrial *ND3* and *ATPase6* genes. *Oncorhynchus mykiss* (rainbow trout) was used as the outgroup. All alignment gaps were counted as missing data. Numbers indicate the percent that each node was retained in 500 bootstrap samplings of the data (parsimony bootstrap percentages above each node, Jukes–Cantor/neighbor-joining percentages below each node).

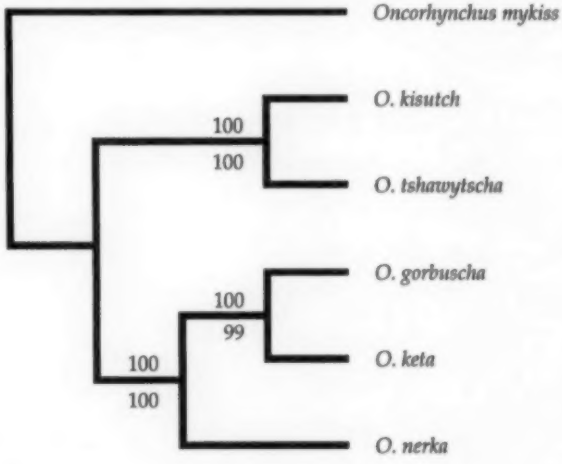

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Table 3. Jukes–Cantor distances and associated standard errors among seven *Oncorhynchus* species based on combined data from *ITS1* and *ITS2* including 928 of 971 bp.

	Sockeye	Chum	Pink	Coho	Chinook	Masu	Rainbow
Sockeye	—	0.0231	0.0231	0.0356	0.0419	0.0406	0.0381
Chum	0.0053	—	0.0170	0.0343	0.0419	0.0406	0.0393
Pink	0.0053	0.0045	—	0.0406	0.0482	0.0444	0.0431
Coho	0.0066	0.0065	0.0071	—	0.0256	0.0386	0.0343
Chinook	0.0072	0.0072	0.0078	0.0056	—	0.0406	0.0331
Masu	0.0071	0.0071	0.0075	0.0068	0.0071	—	0.0268
Rainbow	0.0069	0.0070	0.0073	0.0065	0.0064	0.0057	—

Note: All alignment gaps were excluded in the distance measurement. Distances are given above the diagonal, and standard errors are given below the diagonal.

Table 4. Phylogenetically informative sites in the combined data set.

	Nuclear genes						Mitochondrial genes			
	ITS1	*ITS2*	*GH2C*	*GH2D*	*GH1C*	Total	*ND3*	*ATPase6*	Total	Grand total
Phylogenetically informative sites (PIS)	17	9	7	14	12	59	31	54	85	143
Total aligned sites (TAS)	555*	378	528	1327	714	3502	351	512	863	4365
PIS/TAS	0.031	0.024	0.013	0.011	0.017	0.017	0.088	0.105	0.098	0.033

*This is excluding a region of uncertain homology between bp 544 and 586 (see Fig. 1).

Table 5. Jukes–Cantor distances and associated standard errors among six *Oncorhynchus* species based on the combined data set of 4418 bp of nuclear and mitochondrial DNA sequence data.

	Sockeye	Chum	Pink	Coho	Chinook	Rainbow
Sockeye	—	0.0418	0.0379	0.0483	0.0449	0.0489
Chum	0.0034	—	0.0309	0.0540	0.0500	0.0546
Pink	0.0032	0.0029	—	0.0569	0.0506	0.0586
Coho	0.0037	0.0039	0.0040	—	0.0321	0.0503
Chinook	0.0035	0.0037	0.0038	0.0030	—	0.0427
Rainbow	0.0037	0.0039	0.0041	0.0038	0.0034	—

Note: All alignment gaps were excluded in the distance measurement. Distances are given above the diagonal, and standard errors are given below the diagonal.

Jukes–Cantor genetic distances were calculated (Table 5), and a tree was generated with the neighbor joining aligorithm (MEGA; Kumar et al. 1993) that had the same topology as the tree obtained using maximum parsimony.

Discussion

Molecular evolution of rDNA spacers and GH introns

The rDNA internal transcribed spacers provide excellent nuclear regions for investigating phylogenetic relationships among Pacific salmon. When alignment gaps were exluded from the analysis, the percentage sequence divergence varied from 1.62 to 8.29 between different taxa in the genus *Oncorhynchus*. Growth hormone introns appear to be evolving more slowly with maximum sequence divergence between introns of different species of 2.5%.

Intraindividual sequence variation has been shown to occur in the ribosomal internal transcribed spacers of tiger beetles (*Cincindela dorsalis*) (Vogler and DeSalle 1994) and lake trout (*Salvelinus namaycush*) (Zhuo et al. 1994) but not in the Pacific salmon. This difference may be due to the fact that the technique used in the present study was not as sensitive as cloning for finding polymorphisms, since sequencing was done directly from PCR products. However, if the polymorphisms were equally represented in the PCR product they would have been visible on the autoradiographs. Different sequence variants may be derived from rDNA repeats located on different chromosomes (Vogler and DeSalle 1994). The lack of intraindividual polymorphism in rDNA in Pacific salmon may be a consequence of the nucleolar organizer regions (NORs) in Pacific salmon being restricted to a single locus (Phillips et al. 1986), compared with lake trout, which has NORs at multiple chromosomal locations (reviewed in Phillips et al. 1989).

Phylogenetic analysis of DNA sequence data

The number of phylogenetically informative sites for the relationships among pink, chum, and sockeye for each part of the combined data set is shown in Table 4. There are fewer sites for the *ITS1* and *ITS2* than described earlier, because masu salmon was not included in the combined data. There were more informative sites in the data from mitochondrial genes than from nuclear genes, and the ratio of phylogenetically informative sites to total sites was twice as high for rDNA spacers as for growth hormone introns. Both nuclear data sets agree with those of mtDNA RFLPs (Thomas et al. 1986), mtDNA sequence (Domanico and Phillips 1995), SINEs (Murata et al. 1993), and life histories (Stearley and Smith 1993), which place pink and chum as sister species instead of pink and sockeye as shown by allozymes (Utter et al. 1973) and morphology

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(Smith and Stearley 1989). With the nuclear sequence data and mitochondrial DNA data (Thomas et al. 1986; Domanico and Phillips 1995) in agreement, there is no support for introgression of mtDNA between pink and chum as suggested by Smith (1992).

The pink, sockeye, and chum lineages appear to have undergone rapid separation and evolved independently for some time, making the assignment of sister species difficult. Evidence that each of these species evolved independently for nearly equal times comes from sequence analysis of *GH2C* (Domanico 1994). Pink, sockeye, and chum salmon each had several unique polymorphisms, but there were very few phylogenetically informative sites that provided evidence for a sister relationship between any of these species. Rapid chromosomal evolution also occurred resulting in different karyoypes for each species ($2n = 52$ for pink, $2n = 57–58$ for sockeye, and $2n = 74$ for chum) (reviewed in Hartley 1987).

Reexamination of the data sets that produced trees supporting a sister relationship between pink salmon and sockeye salmon revealed that these trees were weakly supported. In the tree based on data from RFLPs of the of rDNA (Phillips et al. 1992), there was only one synapomorphy between pink and sockeye, and it was an *Apa*I site in the highly variable *IGS* region. *Apa*I recognizes CCCGGG, and sequencing of the spacer regions of the rDNA has revealed intraspecific variation in runs of Cs and Gs. In recent phylogenetic analyses utilizing RFLPs of rDNA, this enzyme has been eliminated (Phillips et al. 1995*a*). In the tree generated from allozyme data (Utter et al. 1973), 12 polymorphic loci were found among all species examined, and the only locus for which pink and sockeye shared a fixed allele was an undescribed muscle protein. In the cladogram based on morphology (Stearley 1992), four synapmorphies were listed for the pink and sockeye clade. Two of these are age-or size-specific, and two (number of gill rakers and tooth size) can be influenced by trophic adaptation.

In conclusion, analysis of the combined sequence data from mitochondrial DNA, nuclear rDNA spacers, and three nuclear growth hormone introns strongly supports a sister relationship of pink and chum salmon as well as between coho and chinook salmon.

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The response of the thermal stratification of South Bay (Lake Huron) to climatic variability

Jacquelynne R. King, Brian J. Shuter, and Ann P. Zimmerman

Abstract: The 37-year record (1955–1992) of water temperature profiles and corresponding meteorology for South Bay (Lake Huron) provide an extensive empirical data set for the study of climate variability and corresponding alterations in lake thermal structure. Thermoclines became shallower over this period and epilimnetic temperatures increased. Canonical analyses correlated warm May–July air temperatures and high July–August solar radiation with warmer epilimnia, larger thermal gradients across the thermocline, and shallower thermoclines. Multivariate canonical scores indicate that these thermal responses have increased in parallel with the recent warming trend since the mid-1960s. Anomalous multivariate scores correspond to El Niño events, and an extreme low score corresponds to the Mount Pinatubo cooling effect of 1992.

Résumé : Les 37 ans d'archives (1955–1992) portant sur les profils de température de l'eau et les données météorologiques correspondantes pour la baie South (lac Huron) fournissent un vaste ensemble de données empiriques pour étudier la variabilité du climat et les modifications correspondantes dans la structure thermique du lac. Les thermoclines sont devenues moins profondes au cours de cette période et les températures épilimnétiques ont augmenté. Des analyses canoniques ont corrélé les températures chaudes de l'air de mai à juillet et le rayonnement solaire intense de juillet à août avec des épilimnions plus chauds, des gradients thermiques plus grands de part et d'autre de la thermocline et des thermoclines moins profondes. Les valeurs canoniques multivariées indiquent que ces réponses thermiques ont augmenté parallèlement avec la tendance au réchauffement qui se manifeste depuis le milieu des années 60. Des valeurs multivariées anormales correspondent aux événements liés au El Niño et une valeur extrêmement faible correspond à l'effet de refroidissement exercé par l'éruption du Pinatubo de 1992.
[Traduit par la Rédaction]

Introduction

There has been considerable concern that a global warming trend will impact on forests (Hoffman 1984), freshwaters (Sanderson 1987), and fisheries (Kendall 1990). Limnologists have given attention to a warming trend's impact on freshwater ecosystems (Carpenter et al. 1992), where effects will likely be directly manifested as changes in thermal structure (e.g., maximum depth of thermocline, thermal gradient across the thermocline, temperature ranges in the epilimnion or hypolimnion) with indirect impacts on biota. One way of estimating the potential magnitude and direction of these changes and their effects on biota, is to look for historical patterns.

A lake's thermal structure is driven by a suite of variables ranging from lake morphometry (Arai 1981; Shuter et al. 1983; Hanna 1990), to lake transparency (Yan 1983; Mazumder et al. 1990; Mazumder and Taylor 1994), through to meteorological conditions (McCormick 1990; Robertson and Ragotzkie 1990; Schindler et al. 1990). The thermal structure of a particular lake can be described on an annual basis by such variables as the time of onset of stratification, thermocline depth, mean epilimnetic temperature, and turnover date. Because thermal structure is important to a lake's productivity (Hutchinson 1957; Wetzel 1975), thermal stratification will influence energy budgets, nutrient recycling, and primary production. Therefore, yearly variation in thermal structure has biological implications. A change in the date of the onset of stratification or turnover may affect the timing of biological events that occur in spring or fall (e.g., hatching or spawning). A change in the duration of stratification has implications for late-summer hypolimnetic oxygen levels. Changes in thermocline depth will affect the thermal habitat available for cold- and warm-water species.

Numerous models have been developed to predict aspects of thermal structure. Given that morphometry determines exposure to solar radiation, air–water heat exchange, and potential for turbulent transport, these models are often morphometrically based (Schindler 1971; Hutchinson 1957; Shuter et al. 1983; Hanna 1990). However, unless water levels change drastically, lake morphometry varies little from year to year so morphometric models have limited use in examining interannual variation in thermal structure within a lake. Water clarity also influences variability in stratification (Yan 1983; Mazumder et al. 1990; Schindler et al.1990, 1996*a*, 1996*b*). Recently, Mazumder and Taylor (1994) examined the relationship between water clarity and thermal structure and found that Secchi depth was strongly correlated with mixing depth in small lakes (<12.5 km^2) but that its relative importance (as determined by r^2 values) was weak in large lakes (12.5–312.5 km^2). In large lakes, unless water clarity changes drastically, interannual variation in thermal structure will likely be driven by changes in meteorological conditions.

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J.R. King[1] **and A.P. Zimmerman.** Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, ON M5S 3G5, Canada.
B.J. Shuter. Ontario Ministry of Natural Resources, Aquatic Ecosystems Research Section, and Aquatic Ecology Group, Department of Zoology, University of Toronto, Toronto, ON M5S 3G5, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: king@zoo.utoronto.ca

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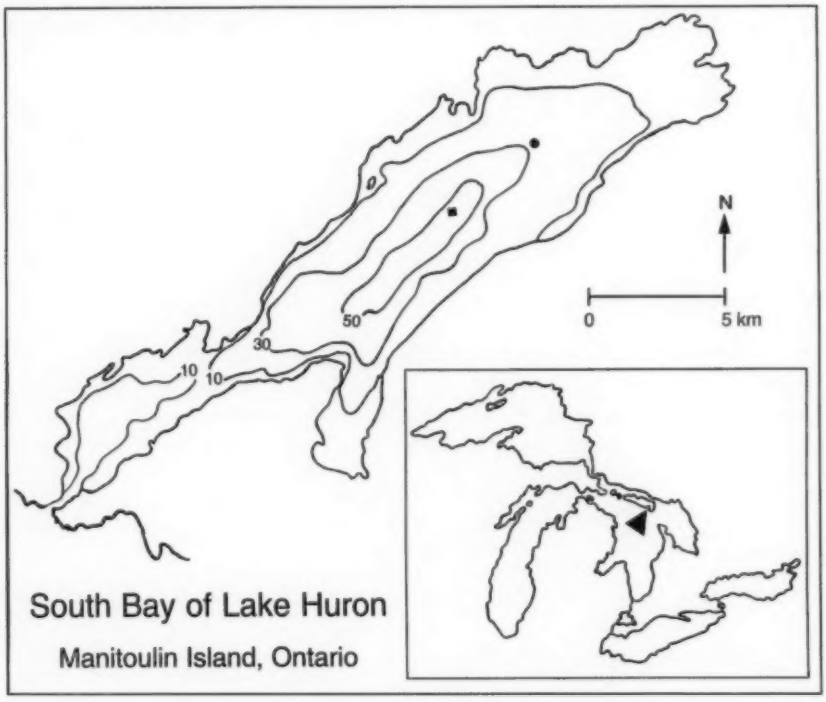
Fig. 1. Bathymetric map (depths in meters) of South Bay of Lake Huron showing the deep (■) and shallow (●) sampling sites. In the inset, the arrowhead indicates the location of South Bay on Manitoulin Island.



Models that describe thermal response to changing climatic conditions are often mechanistic models simulating complex physical processes (Henderson-Sellers 1988; Hondzo and Stefan 1993; McCormick 1990; Blumberg and DiToro 1990; Ford and Stefan 1980; Robertson and Ragotzkie 1990). These models forecast thermal structure throughout a season based on initial lake temperatures and ambient meteorological conditions and may be used to predict long-term changes using simulated meteorological data predicted by general circulation models. These are usually developed for specific lakes and most require calibration with 1 or 2 years of observed lake temperature and meteorological variables. These models often require lake-specific coefficients, for example, eddy diffusivities or turbulence parameters (Aldama et al. 1989), or very fine spatial and temporal resolution of the initial conditions. These can be drawbacks if data are unavailable or too time consuming and costly to compile.

Another approach is to empirically examine the historical correlation between a lake's thermal structure and past meteorological conditions to determine the statistical links between thermal stratification and meteorology. This approach does not require lake-specific coefficients or the simulation of complex physical processes. However, it does require a long-term record of lake temperatures and associated meteorology. Such data sets are rare. Some empirical studies have used extensive records of up to 59 years (Robertson and Ragotzkie 1990); others were limited to about 20 years worth of data (Rodgers 1987; Schindler et al. 1990, 1996*a*), and some have used as few as 2 years (Hondzo and Stefan 1993) to infer statistical links. Robertson and Ragotzkie (1990) used an empirical and a mechanistic modeling approach, and their comparison suggests that both approaches produce similar results. The drawback of historical data analysis is that it relies on uncontrolled natural variation to provide some insight into the sensitivity of the studied system. The correlation of natural variation to change may reflect a direct causal link or it may be an indirect reflection of some other causality in the system. In this paper, we present water temperature and meteorological data for South Bay (Lake Huron; 45°38′N, 81°51′W) for the period from 1955 to 1992. We use multivariate analysis to describe the historical patterns of covariation evident in these two data sets.

Site location

South Bay extends 26 km along a northeast–southwest axis from Lake Huron into the southwestern end of Manitoulin Island (Fig. 1). The bay is connected to Lake Huron by a narrow (<200 m wide) channel. The bay is divided into two distinct basins: an outer and an inner basin. The outer basin receives cold water from Lake Huron during the summer stratification season, and therefore, this study focuses on the inner basin. The inner basin has a mean depth of 16 m, a maximum depth of 58.5 m, a surface area of 73.20 km^2, and maximum dimensions of 18.8 by 5.8 km.

Over the study period (1955–1992), interannual variation in water levels was small and, therefore, unlikely to affect thermal structure as a result of changes in morphometry. Water clarity data for South Bay were not available for the study period; however, other sites on the Great Lakes have exhibited both declines (due to eutrophication) and increases (due to

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zebra mussel (*Dreissena polymorpha*) effects) in water clarity. At South Bay, there was little shoreline development over the time period and zebra mussels had yet to invade. These factors, in combination with the bay's size, makes us reasonably confident that interannual variation in thermal structure at South Bay over the study period was generated by changes in meteorological conditions rather than changes in water clarity.

Methods

Lake temperature and stratification data sets

For the period 1955–1992, personnel from the Ontario Ministry of Natural Resource's South Baymouth Fisheries Research Station collected lake temperature profiles at sampling stations along the 26.7 km fetch of South Bay. South Bay water temperature collection methodology is outlined by McCombie (1967). The profiles were collected at varying intervals ranging from weekly to monthly during the ice-free season. Each profile was collected with a bathythermograph (see Spilhaus 1937 for an instrument description), which was calibrated with each use by a mercury thermometer measurement of the temperature of surface water collected in a bucket. Instruments that showed drift were returned to the manufacturer for repair. The bathythermographs were accurate to ±0.5°C. Permanent photographic records of the thermograph traces were made with the date, location, and bucket surface temperature recorded on each photo.

Traces for two sites from the inner basin were digitized: a deep site (North Sims, 50 m) and a shallow site (Black Rock, 25 m). The digitized vector data were converted into depth and temperature values using an algorithm that corrected for any discrepancies between the bucket and bathythermograph surface temperatures and converted the values to metric scales when required. Random profiles were checked for agreement between computer-generated data and data read manually from the original photos.

The raw temperature–depth data for the deep and shallow sites were used to classify the thermal structure for each year. Reduced sampling efforts in 1985–1989 produced insufficient data for the shallow site for these years. There were insufficient deep-site data for 1985 only. As a result, there were 37 years of data for the deep site in two periods (1955–1984 and 1986–1992) and 33 years of data for the shallow site in two periods (1955–1984 and 1990–1992). Approximately 43% of the 37 years of the deep-site data were sampled weekly, 38% bimonthly, and 19% monthly. For the shallow site, 48% of the data were sampled weekly, 43% bimonthly, and 9% monthly.

Stratification profiles were fitted to a logistic equation using the SAS NLIN procedure (SAS Institute Inc. 1989). The thermocline depth was mathematically defined as the depth where the second derivative of the fitted equation equaled zero. Random profiles were checked for agreement between calculated thermoclines and manually estimated thermoclines. To ensure consistency of interpretation from profile to profile and from year to year, profiles exhibiting double thermoclines were characterized using the deepest thermocline only.

The yearly thermal structure was characterized by 10 stratification variables. The seasonal thermocline depth was calculated as the mean depth of all profiles for the stratified period and the midsummer thermocline depth was calculated as the mean depth for July and August. The standard deviation associated with the seasonal thermocline depth was used as a surrogate for thermocline migration. The epilimnetic temperature and hypolimnetic temperature were calculated as the ice-free period's mean temperature at 1 m below the surface and 1 m above the bottom, respectively. The maximum difference between corresponding epilimnetic and hypolimnetic temperatures was calculated for the season. We defined the epilimnetic and hypolimnetic layers as the top and bottom layers in which change in temperature was <1°C per meter and used epilimnetic stability and hypolimnetic stability as the mean differences in temperature within each of these layers for the ice-free period. The onset date of stratification was defined as the day of the year when the difference between the epilimnetic and hypolimnetic temperatures was ≥3°C. Over several years, this temperature difference was the threshold difference above which a developing thermocline remained in the water column and did not degrade with the occurrence of a strong wind event. Our estimate is consistent with other definitions for the presence of stratification. Robertson and Ragotzkie (1990) considered a lake to be stratified when the difference in temperature from the surface to bottom was ≥2°C and Yan (1983) used a cutoff difference ≥4°C. The opposite criterion (temperature difference ≤3°C) was used to define the turnover date. These dates were estimated by interpolation between observed profiles or between ice-out or freeze-up dates (when the difference in temperature was assumed to be zero) and the next observed profile. Time series for each stratification variable at the deep site are presented in Fig. 2. The shallow site's stratification variables had a similar pattern of variability.

Meteorological data

Meteorological data collection at South Bay began in 1947. Hourly air temperature (°C), wind speed ($km \cdot h^{-1}$) and prevailing wind direction were obtained from the South Baymouth meteorological station through the Ontario Weather Archives of the Atmospheric Environment Service of Environment Canada. Hourly incoming solar radiation ($MJ \cdot m^{-2}$) for Manitoulin Island were obtained from the Industrial Climate Research Group of Environment Canada. The day of the year of ice-out data were obtained from the Ontario Ministry of Natural Resources.

The monthly means of air temperature, solar radiation, and wind speed, the monthly prevailing wind direction, and the percentage of time the wind blows along the fetch during a month were calculated for April–November. The ice-out date was included in the meteorological data set as a summary variable of the weather conditions just prior to ice-out. Ice-out date also signifies the date when the physical barrier of ice is removed and the water surface is more completely exposed to meteorological conditions.

Using monthly data from April to November, the meteorological data matrix had 37 observations and 36 variables. For reasonable stability and reliability of the outcome of any statistical analysis, the general goal is to use a data matrix with a 3:1 ratio of observations to variables. For South Bay, that means that no more than 12 meteorological variables should be used. Variable reduction was achieved by pooling the monthly data into longer, seasonal periods and by focusing on those variables that contributed most to the overall variation in the data set.

A principal components analysis (PCA) was performed on a correlation matrix of all meteorological variables using the PRINCOMP procedure in SAS (SAS Institute Inc. 1989) to identify the months and variables that contributed the most variation over the 37 years. Joliffe (1972) suggests that a reasonable method of identifying representative variables is to perform a PCA on all 36 variables and, beginning with the first principal component, to select the highest loading variable from each significant component. Here, significant principal components were selected based on Frontier's (1976) broken stick model because it was identified by Jackson (1993) as a consistent approach for determining a suitable number of components for interpretation.

From the PCA on the 36 meteorological variable data set, we selected 7 variables that contributed the most variation: ice-out date, May and July air temperatures, October solar radiation, May wind speed and wind direction, and July wind along the fetch. As the preliminary screening suggested useful parameters across May–October, we sectioned this season into three periods: early (May–June), middle (July–August), and late (September–October). Late-season meteorological variables are included in the analyses because fall aspects of

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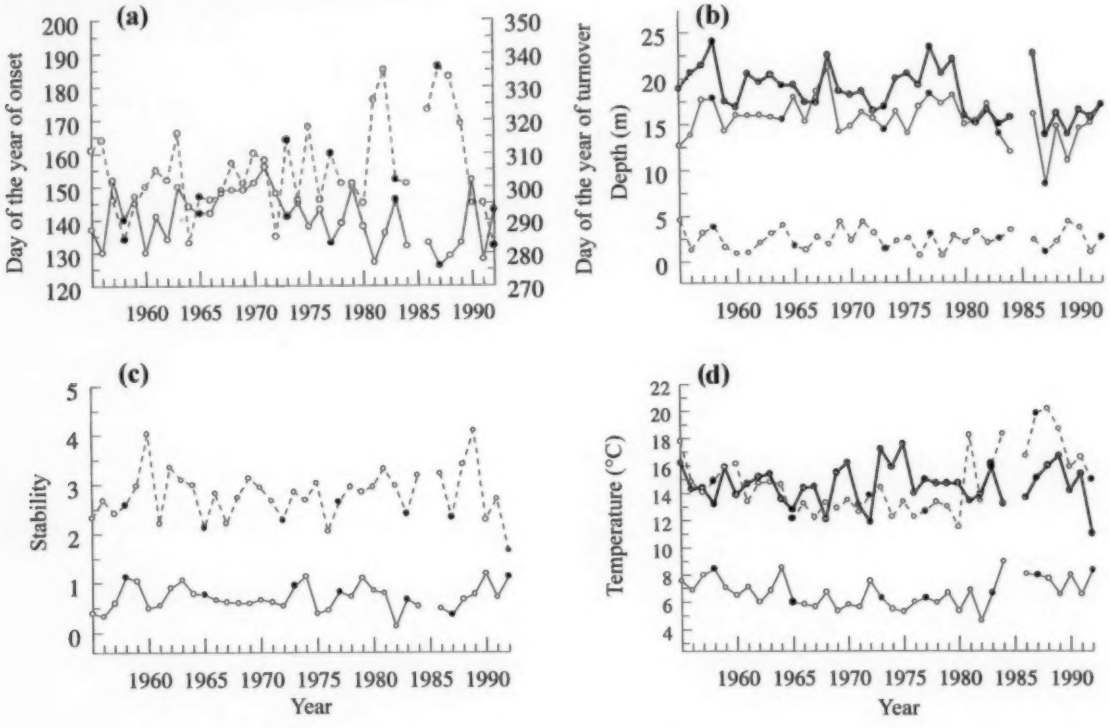
Fig. 2. Line plots of stratification variables for the deep site from 1955 to 1992 with the missing year (1985) indicated by a break in the line. Closed circles for all plots indicate El Niño years (1958, 1965, 1973, 1977, 1983, and 1987) and Mount Pinatubo cooling year (1992). (*a*) Onset day of the year of stratification (solid line) and fall turnover day of the year (dotted line). (*b*) Mean depth (m) of seasonal thermocline (thick line) and midsummer thermocline (thin line) along with amount of seasonal thermocline migration (m) (broken line). (*c*) Mean stability number of epilimnion (solid line) and hypolimnion (broken line), where a stability number close to zero indicates a stable layer that is approximately isothermal. (*d*) Mean temperature (°C) of the epilimnion (broken line) and hypolimnion (thin line) and the maximum temperature difference between the two layers (thick line).



thermal structure (e.g., turnover date) are included in the stratification data set.

We selected wind speed, rather than prevailing direction or percentage of wind along the fetch, to retain because we felt it was most likely to be available for other studies, and its inclusion would be useful for comparisons. Ice-out, air temperature, and solar radiation were also retained. Hence, the meteorological data were summarized by 10 variables: ice-out date and the early (May–June), middle (July–August), and late (September–October) means for air temperature, solar radiation, and wind speed (Fig. 3).

Data analysis

All variables were screened prior to analysis for outliers, normality, and homoscedascity and were transformed as required. Correlation analyses between each of the deep-site stratification variables and the meteorological variables were used to identify any significant trends between years. We omitted 1992 data from our trend analyses because the Mount Pinatubo eruption (June 11, 1991) produced a large, transitory global cooling effect throughout 1992 (Shine et al. 1996). Inclusion of data for this year in a trend analysis would likely overwhelm the presence of smaller but evident trends from earlier years.

Canonical correlation analysis (CCA) was introduced by Hotelling (1936) as a method of maximally correlating a linear combination (canonical axis) of dependent variables with a linear combination of independent variables. Canonical correlation analysis is a useful tool for exploring overlapping variation between two data sets. We chose CCA as the appropriate tool for analysis of the South Bay data set as we believe that meteorological and stratification variables vary in a dependent or combined fashion. As with other multivariate techniques, CCA also provides a useful method of expressing the variation across several dimensions in a single, or at least fewer, dimension(s). Two CCAs (one for the deep and one for the shallow site) were performed between the meteorological and the stratification data sets using the CANCORR procedure in SAS (SAS Institute Inc.1989). Paired *t* tests between the deep and shallow site stratification variables were used to assess differences in thermal structures between sites.

Results

General trends

Correlation analysis indicates that seasonal thermocline depth, epilimnetic temperature, and turnover date have shown significant ($p < 0.05$) trends over the period of 1955–1991 with thermoclines becoming shallower ($r = -0.48$), epilimnetic temperatures increasing ($r = 0.35$), and turnover events occurring later in the fall ($r = 0.38$). Schindler et al. (1996*a*) reported deepening of midsummer thermoclines for their study period 1970–1990, and in South Bay for this same period, midsummer thermoclines became shallower ($r = -0.46$; $p < 0.05$).

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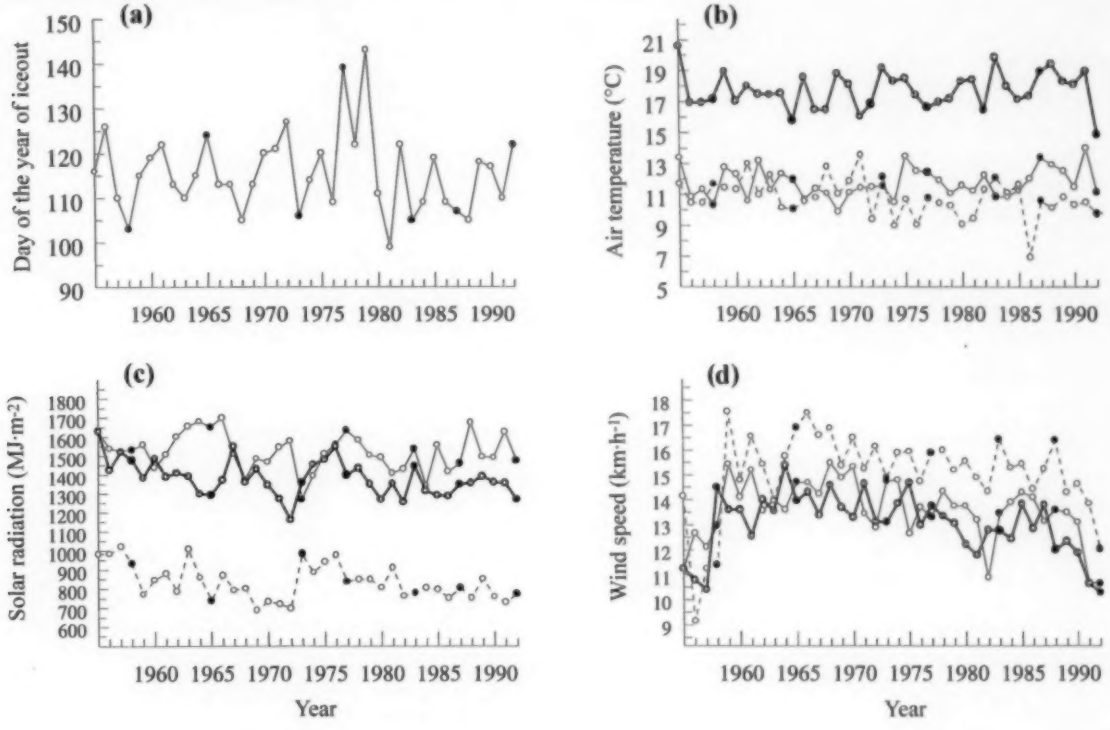
Fig. 3. Line plots of meteorological variables over the study period 1955–1992. Closed circles for all plots indicate El Niño years (1958, 1965, 1973, 1977, 1983, and 1987) and Mount Pinatubo cooling year (1992). (*a*) Day of the year of ice-out. (*b*) Mean air temperatures (°C). (*c*) Mean incoming solar radiation ($MJ \cdot m^{-2}$). (*d*) Mean wind speed ($km \cdot h^{-1}$). For all plots, except ice-out date, early season is the thin line, midseason is the thick line, and late season is the broken line.



From 1955–1991, late-season air temperatures actually decreased ($r = -0.33$; $p < 0.05$), and middle- and late-season solar radiation decreased ($r = -041$ and -0.42 respectively; $p < 0.05$). It is interesting to note that, while late-season air temperatures decreased, early ($r = 0.22$, $p = 0.19$) and middle season ($r = 0.20$, $p = 0.23$) air temperatures increased from 1955 to 1991, though these trends were not significant. Schindler et al. (1990) reported increases in wind speed for 1969–1988, attributed to increased wind exposure with forest loss due to fire. Figure 3*d* suggests that South Bay wind speed has exhibited a decline from 1960 to 1991, and correlation analyses for this period indicates a significant ($p < 0.05$) decrease in wind speed for the early ($r = -0.55$), middle ($r = -0.67$), and late ($r = -0.48$) season.

Stratification and meteorology canonical correlation

Canonical correlation analysis between the meteorological data set and the deep site stratification data set produced a first canonical pair of axes (a meteorology axis and a stratification axis) explaining 48.73% of overlapping variance (canonical correlation of 0.95 signifying that the meteorology canonical axis is highly correlated with the stratification canonical axis) and a second canonical pair of axes explaining 31.45% of variance (canonical correlation of 0.92). Chi-square tests indicate that significant overlapping variance between the two sets of variables are expressed only by the first two canonical pairs of axes (Table 1).

These results can be interpreted using the correlation between observed variables (both meteorological and stratification) and their respective new canonical axes (Table 1). If we focus on variables with correlations that exceed a value of 0.3, then we ensure that at least 10% of the observed variation in each variable is accounted for by its canonical axis. Using this admittedly arbitrary but useful cutoff value, the first canonical pair of axes for the deep site correlates meteorological conditions characterized by warm early and middle season air temperatures, high midseason solar radiation, and a relatively calm early season with thermal regimes characterized by earlier stratification onset dates, shallower seasonal and midsummer thermocline depths, warmer epilimnia, and larger maximum temperature differences between the epi- and hypo-limnia. The second canonical pair of axes for the deep site reinforce the pattern of covariation: warm midseason air temperatures and high solar radiation (late season) but with stronger early season wind speeds correlated with shallower midsummer thermoclines, larger maximum temperature differences, and also a more unstable hypolimnion (Table 1).

Canonical correlation analysis performed among the 10 variables of the meteorological data set and the 10 stratification variables for the shallow site produced a first canonical pair of axes explaining 67.29% of overlapping variance (canonical correlation of 0.96). Chi-square tests indicated that only the first canonical variate pair of axes represent significant overlapping variance (Table 1). Interpretation of these results

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Table 1. Correlations between observed variables and their respective canonical axis, chi-square and p values for significance, overall canonical correlation between axes, and redundancy values for the deep and shallow site.

	Deep site		Shallow site, first pair of canonical axes $\chi^2_{(100)} = 149.68$ ($p < 0.05$)
	First pair of canonical axes $\chi^2_{(100)} = 169.99$ ($p < 0.001$)	Second pair of canonical axes $\chi^2_{(81)} = 112.13$ ($p < 0.05$)	
Meteorological data set			
Ice-out date[a,x]	0.24	0.10	0.19
Early air temperature	0.54	0.04	0.50
Middle air temperature	0.80	0.30	0.77
Late air temperature	0.12	0.02	0.20
Early solar radiation	0.17	-0.08	0.09
Middle solar radiation	0.57	0.07	0.54
Late solar radiation	0.15	0.37	0.41
Early wind speed[c,z]	0.32	0.52	0.14
Middle wind speed	0.28	0.33	0.26
Late wind speed[c,z]	0.05	0.03	0.18
Redundancy	0.15	0.05	0.14
Stratification data set			
Onset date	0.34	0.06	0.30
Turnover date[a]	0.27	0.18	0.37
Seasonal thermocline depth[a,y]	0.32	0.28	0.21
Midsummer thermocline[x]	0.57	0.32	0.45
Seasonal thermocline migration[b]	0.03	0.15	0.24
Epilimnetic temperature[a,x]	0.52	0.01	0.52
Hypolimnetic temperature[a,x]	0.02	0.02	0.10
Maximum temp difference	0.72	0.51	0.72
Epilimnetic stability	0.22	0.15	0.09
Hypolimnetic stability[b,x]	0.13	0.46	0.22
Redundancy	0.14	0.06	0.13
Canonical correlation	0.95	0.92	0.96

[a]Deep site values were log transformed.
[b]Deep site values were square-root transformed.
[c]Deep site values were squared transformed.
[x]Shallow site values were log transformed.
[y]Shallow site values were square-root transformed.
[z]Shallow site values were squared transform

generates a similar picture to that obtained from the deep site analysis: warm early and middle season air temperatures and high middle and late season solar radiation correlated with earlier onset dates, later turnover dates, shallower midsummer thermoclines, warmer epilimnia, and larger maximum temperature differences between the epilimnion and hypolimnion.

Redundancy values (Table 1) give the average proportion of variation in one data set that is explained by the new canonical axis in the other data set (e.g., in the deep site's analysis, 14% of the stratification data set's variation is explained by the meteorology canonical axis). These values provide one measure of strength of the covariation between the two data sets. However, redundancy values are influenced by the number of variables included in the overall analysis. We re-analyzed the deep site data, using an objective variable reduction procedure to improve redundancy values. Specifically, we used backward stepwise CCA (Thompson 1984) with variable selection based on interset communalities (Gittins 1985). After the number of meteorological and stratification variables were reduced from 10 and 10 to 3 and 4, respectively, the meteorological and stratification redundancy values for the first canonical axis increased from low, initial values of 15 and 14% to respectable values of 37 and 40%. Further increases in redundancy might require collection of both stratification and meteorological data at a much finer (e.g., daily) time scale. The re-analysis did not change the basic pattern of covariation between meteorological and stratification data. The first pair of canonical axes ($\chi^2_{(12)} = 55.19$; $p < 0.005$; canonical correlation of 0.86 and 88% overlapping variance) correlated warmer early and middle season air temperatures and higher midseason solar radiation (meteorological axis correlations: 0.50, 0.96, 0.35, respectively) with shallower seasonal thermoclines, shallower midsummer thermoclines, warmer epilimnetic temperatures, and larger maximum temperature differences between epi- and hypo-limnia (stratification axis correlations: 0.45, 0.75, 0.60, 0.87, respectively).

Differences between the deep and shallow sites

Mean values (Table 2) for each of the stratification variables for both the deep and shallow sites were compared using paired t tests. Significant differences ($p < 0.05$) indicated that the deep site turns over later than the shallow site, the deep site has

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a deeper seasonal thermocline that migrates less than the thermocline at the shallow site, and the deep site has a hypolimnion that is colder but also less stable than the hypolimnion at the shallow site.

Discussion

There is a distinct historical pattern of covariance between thermal stratification and meteorological variability in South Bay as indicated by the similarities between the deep and shallow sites' first canonical axes. The correlation between original variables and the first canonical axes for both sites are summarized below to include only variables with correlations ≥0.3; the direction of each correlation is specified by the positive or negative sign appearing in front of each variable; the numbers in brackets order the variables' correlations by absolute value from largest to smallest:

	Deep site	Shallow site
Meteorological variables	+ Early (May–June) air temperature (3) + Middle (July–August) air temperature (1) + Middle solar radiation (2) – Early wind speed (4)	+ Early (May–June) air temperature (3) + Middle (July–August) air temperature (1) + Middle solar radiation (2) + Late (September–October) solar radiation (4)
Stratification variables	– Onset date (4) – Seasonal thermocline (5) – Midsummer thermocline (2) + Epilimnetic temperature (3) + Maximum temperature difference (1)	– Onset date (5) + Turnover date (4) – Midsummer thermocline (2) + Epilimnetic temperature (3) + Maximum temperature difference (1)

The three meteorological variables with the highest correlations to the meteorology axis are the same for the deep and the shallow sites (early and midseason air temperatures, midseason solar radiation). The three stratification variables with the largest correlations are also the same for the two sites (midsummer thermocline depth, epilimnetic temperature, maximum difference in epilimnetic and hypolimnetic temperatures). The directions of correlation of all meteorological and stratification variables (indicated by a positive or negative correlation to their respective axis) are the same for both sites (e.g., ice-out date is negatively correlated with the first canonical meteorology axis for both the deep and the shallow site). If variables that correlate highly for both sites are expressed as a general relationship for South Bay, years with warm early and midseason air temperatures and high midseason solar radiation also have early onsets of stratification, warmer epilimnetic temperatures, larger maximum temperature differences between epi- and hypo-limnia, and shallower midsummer thermoclines. There are differences between the two sites' canonical relationships with respect to the relative correlations of variables, but this is an expression of the differences in the means of turnover date, seasonal thermocline depth and migration, and hypolimnetic temperature and stability.

Table 2. Paired *t*-test results between stratification variables for the deep and shallow sites; *t* values and corresponding *p* values are indicated along with the mean for each site.

			Mean	
Variable	*t*	*p*	Deep site	Shallow site
Onset date	1.40	0.1670	143	141
Turnover date	5.41	<0.0001	305	289
Seasonal thermocline depth	2.78	<0.0001	18.6	17.0
Midsummer thermocline depth	0.46	0.6435	15.6	15.3
Seasonal thermocline migration	10.18	<0.0001	2.5	5.8
Epilimnetic temperature	1.08	0.2847	14.8	14.3
Hypolimnetic temperature	2.49	0.0189	6.7	7.3
Maximum temperature difference	1.48	0.1431	14.6	14.1
Epilimnetic stability	1.12	0.2654	0.7	0.6
Hypolimnetic stability	4.71	<0.0001	2.8	2.2

The general relationship is a reflection of the correlation between meteorological and stratification variables. While it does not provide definitive causal links, it does provide some insight into overlapping historical covariation. Early season air temperatures most likely influence the date of onset of stratification, epilimnetic temperature, and thermocline depths, while midseason air temperatures and solar radiation would affect only middle and late season epilimnetic temperatures, maximum temperature differences, and thermocline depths. Midseason air temperatures and solar radiation will not affect onset of stratification dates, but because years with warm early air temperatures tend to also have high midseason air temperatures and solar radiation, there is a statistical link between onset dates and these midseason meteorological variables.

Robertson and Ragotzkie (1990) suggested that hypolimnetic temperatures could be expected to change very little or increase only slightly with climatic warming. We found similar results: hypolimnetic temperature did not vary much over the period of 1955–1992 ($r = -0.01$; $p > 0.78$) and was not selected by canonical analysis for either site as a variable highly correlated with overlapping meteorology variance (Tables 1 and 2). However, epilimnetic temperature did increase for our study period and was selected by both canonical correlation analyses. Thus, changes in the maximum difference in temperature largely reflect changes in epilimnetic temperature. This does not imply that maximum difference is a redundant variable. Its association with meteorological conditions may have implications for settling rates of phytoplankton because of less dense water or for migrating organisms (e.g., zooplankton), which would have to endure larger temperature changes as they moved from hypolimnetic to epilimnetic regions.

Multivariate standardized scores along the canonical stratification axis from the deep site's backward-stepwise canonical

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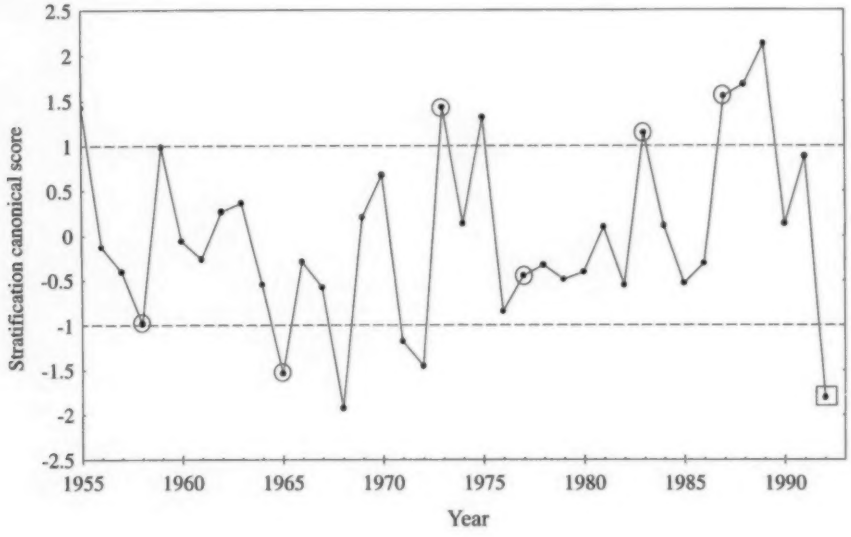
Fig. 4. Standardized stratification canonical scores for the first canonical variate relationship for the deep site. The solid line indicates the mean of zero; broken lines indicate 1 SD above and below the mean. The stratification score for the missing year (1985) was estimated from a canonical climate score calculated with the known climate variables. Large open circles indicate El Niño years; large open square indicates Mount Pinatubo cooling year.



correlation analysis represent the variation of several stratification variables in a single dimension (Fig. 4). Stratification canonical scores greater than zero reflect thermal structures that correlate to years with warmer early and midseason air temperatures and high midseason solar radiation. These thermal structures have warmer epilimnia, larger temperature differences between layers, and shallower thermoclines. Years 1955, 1959, 1973, 1975, 1983, 1987–1989, and 1991 have the 10 highest stratification scores, while more large positive scores occurred over the last 13 years of the time series (1980–1992) than occurred in its first 25 years (1955–1979). This suggests that the frequency of occurence of warmer epilimnia, larger thermal gradients, and shallower thermoclines has increased during the warming trends of the 1980s and 1990s. Each El Niño year (except 1977), exhibited an anomalous stratification score. An extremely low score was observed in 1992, a year that was expected to be cool because of the eruption of Mount Pinatubo. The occurence of these anomalous scores suggests that the stratification time series reflects extreme climate events.

Average global air temperatures have risen 0.3–0.6°C since the mid-19th century to the present (Nicholls et al. 1996). Within our study period (1955–1992) there was a short-term cooling phase (pre-1965) followed by a warming trend that was broken by the Mount Pinatubo anomaly (Nicholls et al. 1996). During the cooling phase (1955–1965) there was a nonsignificant decline ($r = -0.26$; $p > 0.15$) in stratification scores. During the warming period (1966–1991), stratification scores have increased significantly ($r = 0.56$; $p < 0.05$).

Many models predicting thermal response of lakes to a changing climate agree that warmer years will correspond to earlier onsets of stratification, warmer water temperatures (especially in the epilimnion), longer stratification periods, and larger temperature gradients through the water column (Roberston and Ragotzkie 1990; Schindler et al. 1990; Rodgers 1987; McCormick 1990; Blumberg and DiToro 1990; Hondzo and Stefan 1993). The models differ in their predictions of how thermoclines will respond to a warming trend. Robertson and Ragotzkie (1990) suggested almost no change in thermocline depth, while other models forecast shallower thermoclines because of a warming trend. McCormick (1990) simulated Lake Michigan's response to climate warming and observed that, with increasing air temperature, a warmer epilimnion translated into strong thermal and density gradients. This strong density gradient produced a shallower thermocline. Blumberg and DiToro (1990) used hydrodynamic model simulations for Lake Erie and found that weaker winds or large surface heating produced earlier onsets of stratification and shallower thermoclines. For South Bay, while wind speed was not retained in our re-analysis, in the initial canonical correlation analysis it did correlate highly with the deep site's canonical axis and warm years coupled with weaker winds (at least in early summer) were correlated with earlier onset dates and shallower thermoclines. The general response of South Bay's thermal stratification to the change in a suite of meteorological variables parallels the predictions of these two mechanistic models: warmer years will produce shallower thermoclines associated with warmer epilimnia and large thermal and density gradients across the thermocline.

Schindler et al. (1990, 1996*a*, 1996*b*) have predicted that warmer climatic conditions will produce deeper thermoclines in small boreal lakes. For the study period 1970–1990, they observed warmer and drier conditions that were associated with deeper thermoclines. The coupling of warmer air temperatures, increased evaporation, and decreased precipitation with decreased hydrologic inputs, translated into reduced dissolved organic carbon (DOC) inputs. This reduction in DOC inputs increased water clarity and solar penetration. Increased

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solar penetration, combined with increased wind speeds, caused thermoclines to deepen.

Fee et al. (1996) have also observed a strong relationship between reduced DOC concentrations (as a consequence of climate change), increased water clarity, and deeper mixing depths in small lakes. Though they observed a strong relationship between mixing depths and water clarity for lakes <5 km^2, they reported a more variable relationship in larger lakes. They concluded that lower DOC concentrations would cause transmission to increase and result in deeper epilimnia for small lakes but that there would be no similar effect in large lakes. Mazumder and Taylor (1994) examined the relationship between water clarity and mixing depth for a range of lake sizes and found that the relative importance (as indicated by r^2 and p values) of water clarity in determining mixing depth was greater in small lakes (<12.5 km^2). However, regression coefficients for water clarity did not seem to be affected by lake size. Fee *et* al. (1996) point out that Sterner's (1990) analysis of the same data showed that both mixing depth and transparency were positively correlated with lake size and therefore the small correlation between mixing depth and transparency among all lakes greater than 12.5 km^2 could simply reflect the underlying relation with lake size that both variables shared.

In any event, linkage between dry conditions in summer, water clarity, and thermal structure in small lakes likely accounts for the difference in thermocline response between the South Bay and Experimental Lakes Area data sets. Fee et al. (1996) provide a useful recount of a study by Boyce (1974) that examined the scales at which different types of motion that occur in lakes become significant. As light attenuates with depth and mixing depth increases with lake size, there must be a lake size where little solar energy reaches to or past the mixing depth. The increasing intensity and significance (with respect to lake size) of mechanical energy due to winds and surface currents means that solar penetration (controlled by water transparency) in large lakes has little or no bearing on the establishment of a seasonal mixed layer. This supports a model of meteorological conditions, as transferred by wind energy, having a progressively greater influence on thermal structure as lake size increases.

Weather-generated, interannual variation in hydrologic input may have a greater effect on interannual variation in transparency in small lakes because of their smaller volumes of water and generally shorter renewal times. This means that, for any given year, hydrologic variability will greatly affect that year's transparency. However, the same hydrologic variability would translate into small variability in transparency in larger lakes because of their larger volumes and longer renewal times. In large lakes, there would likely be little thermal structure response to small short-term water clarity fluctuations, but there may ultimately be a response to long-term (e.g., decadal) trends in water clarity.

Very large water clarity decreases would be required to account for the observed decreases in thermocline depth at South Bay. Such a decrease could be a result of intense eutrophication; however, shoreline development around South Bay has been minimal for the period 1955–1992, and the shoreline itself is composed mainly of barren rocks, rubble, and limestone. Though long-term water clarity data are not available for South Bay, anecdotal reports by Ministry of Natural Resources personnel suggest that large changes in water clarity due to eutrophication over this period are very unlikely. We do not feel that the interannual variation of the long-term trends in thermal structure have been due to changes in water clarity. The significant overlapping variation of stratification and meteorological variables, the parallel increase of multivariate stratification scores with the post-1965 warming trend, and the evidence of El Niño and Mt. Pinatubo signals in stratification scores indicate that the thermal structure of this large lake is responding to changes in climatic conditions. These correlations imply that warming trends will have consequences for thermal structure. Our results suggest that, for large lake systems like South Bay, climate warming may translate into earlier onset dates of stratification (which will affect the growing season for biota and late-season hypolimnetic oxygen regimes), shallower thermoclines (which will alter thermal habitat volumes), and warmer epilimnia coupled with larger temperature differences in the water column (which will alter the range of temperatures to which migrating and epilimnetic organisms are exposed).

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Internal phosphorus loading in Lake Mendota: response to external loads and weather

P.A. Soranno, S.R. Carpenter, and R.C. Lathrop

Abstract: We quantified summer internal phosphorus (P) loads and all the major P fluxes in Lake Mendota, Wisconsin, a deep stratified eutrophic lake, during two summers of contrasting external loads to determine (*i*) whether internal loading by entrainment (mixing at the thermocline) is a significant part of the epilimnetic P budget and (*ii*) what factors lead to interannual variability in internal loading. We estimated variables for the P budget weekly (standing stock, sedimentation) or daily (outflow, inflow, and entrainment) during a summer of average runoff (1992) and one of higher than average runoff (1993). Entrainment, estimated by calculating the amount of P transported into the epilimnion after the thermocline deepened following storms, was about 10 times higher than external loading during 1992, but was about equal to external loading during 1993. When entrainment was included, the epilimnetic P budget balanced. Interannual variability in internal loading appears to be due to a combination of water column stability, weather, and the P levels accumulated in the metalimnion. External loads to the epilimnion during summer 1993 were much higher than in 1992 (7.0 and 0.9 mg $P \cdot m^{-2} \cdot day^{-1}$, respectively); however, total loads (internal + external) were more similar (12.5 and 7.8 mg $P \cdot m^{-2} \cdot day^{-1}$ in 1993 and 1992, respectively). Although summer chlorophyll concentrations were similar in the two summers, blue-green algal biomass in 1993 was about double that in 1992.

Résumé : Nous avons quantifié les charges internes estivales de phosphore (P) et tous les principaux flux du P dans le lac Mendota, Wisconsin, qui est un lac profond eutrophe stratifié, au cours de deux étés pendant lesquels les charges externes ont contrasté pour déterminer (*i*) si la charge interne par entraînement (mélange au niveau de la thermocline) constitue une partie substantielle du bilan épilimnétique du P et (*ii*) quels facteurs sont responsables de la variabilité interannuelle de la charge interne. Nous avons estimé les variables du bilan du P hebdomadairement (biomasse, sédimentation) ou quotidiennement (flux de sortie, flux d'entrée et entraînement) au cours d'un été où le ruissellement était moyen (1992) et d'un été où le ruissellement était supérieur à la moyenne (1993). L'entraînement, estimé en calculant la quantité de P transportée dans l'épilimnion après que la thermocline s'est enfoncée après des tempêtes, était environ 10 fois plus important que la charge externe en 1992, mais était à peu près égal à cette dernière en 1993. Lorsque l'entraînement était inclus, le bilan du P épilimnétique était équilibré. La variabilité interannuelle de la charge interne semble être due à une combinaison de stabilité de la colonne d'eau, de climat et des concentrations de P accumulées dans le métalimnion. Les charges externes de l'épilimnion au cours de l'été 1993 étaient beaucoup plus élevées qu'en 1992 (7,0 et 0,9 mg $P \cdot m^{-2} \cdot jour^{-1}$, respectivement); toutefois, les charges totales (internes + externes) se ressemblaient davantage (12,5 et 7,8 mg $P \cdot m^{-2} \cdot jour^{-1}$ en 1993 et 1992, respectivement). Bien que les concentrations de chlorophylle estivales étaient semblables au cours des deux étés, la biomasse des algues bleu-vert en 1993 était environ le double de celle de 1992.
[Traduit par la Rédaction]

Introduction

Phosphorus (P) loading from diffuse or nonpoint sources continues to be a large source of nutrients to lakes (NRC 1992; Duda 1993). In culturally eutrophied lakes, many years of high nutrient loads may leave a legacy of stored nutrients in lake sediments (Sas 1989; Cooke et al.1993). This internal store of nutrients can take many years to decline, at rates which depend on the morphometry and hydrologic regime of a lake, sediment minerology, and pore water chemistry (Sas 1989). In both shallow and deep lakes, internal nutrient loading acts as a memory of past external loads and is the reason that lakes often fail to respond to nutrient reduction strategies (NRC 1992).

Internal nutrient loading can be especially high in shallow lakes where mixing at the sediment–water interface effectively maintains nutrients in the water; and depending on climate and external nutrient loading conditions, interannual variability of internal loads can be high (Jensen and Andersen 1992; Sondergaard et al. 1993; van der Molen and Boers 1994). In contrast, internal loading to the epilimnion in deep lakes during the summer can be low, because thermal stratification can effectively isolate much of the sediment area from the epilimnion even though nutrients accumulate in the hypolimnion during anoxia. Because hypolimnetic nutrients at the annual scale are mixed throughout the water column during fall and spring overturn, total internal loading in deep lakes can be quite large. In fact, nutrient dynamics in lakes with anoxic hypolimnia have been found to behave differently than lakes with oxic hypolimnia, partly as a result of this internal loading

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P.A. Soranno[1] and S.R. Carpenter. Center for Limnology, 680 North Park Street, University of Wisconsin, Madison, WI 53706, U.S.A.
R.C. Lathrop. Wisconsin Department of Natural Resources, 1350 Femrite Drive, Madison, WI 53716, U.S.A.

[1] Author to whom all correspondence should be addressed. Present address: Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824, U.S.A. e-mail: soranno@pilot.msu.edu

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(Nürnberg 1984). However, the importance of internal loading that occurs within the summer stratified season alone is not well documented.

Internal loading in stratified lakes during the summer period is complex, difficult to measure, and has only occasionally been quantified directly (Stauffer and Lee 1973; Larsen et al. 1981; Kortmann et al. 1982; Effler et al. 1986; James et al. 1990). This internal loading occurs through two mechanisms: (*i*) release of P from sediments in contact with the epilimnion and (*ii*) turbulent vertical flux across the thermocline when P gradients between the hypolimnion and epilimnion are large (Stauffer and Lee 1973; Kortmann et al. 1982; Effler et al. 1986). In lakes where the epilimnetic volume is large relative to the area of epilimnetic sediments, the first mechanism is minor relative to other fluxes. In such lakes, the thermocline can be a highly dynamic boundary and fluxes across it are potentially large (Stauffer and Lee 1973). Mixing usually results from storms that cause the thermocline to deepen and entrain nutrients from the hypolimnion into the epilimnion. In some lakes, P entrainment into the epilimnion has been shown to be a significant input and has been suggested to exceed external inputs during the summer stratified season (Stauffer and Lee 1973; Larsen et al. 1981; Effler et al. 1986; Stauffer 1987).

At the annual scale, external nutrient loading can be the largest source of nutrients to lakes. In northern temperate climates, most of this loading occurs in winter–spring from a combination of snowmelt and spring runoff, which is strongly driven by weather conditions. In fact, spring runoff or its surrogate, in-lake spring total P (TP), is correlated to average summer algal biomass and in particular, blue-green algal biomass (Lathrop and Carpenter 1992). Nevertheless, variance around these relationships is large (Lathrop and Carpenter 1992; Stow et al. 1997), and consideration of internal loading during the summer may improve predictions. Because few studies have directly measured internal P loads in stratified lakes, it is not known if internal loads contribute a relatively constant source of nutrients year to year or if they vary as widely as external loads do in response to changing weather conditions.

Our objectives in this study were to quantify summer internal P loads and all the major P fluxes in Lake Mendota, a stratified eutrophic lake in Wisconsin, during 2 years of contrasting external loads. Specifically, we wanted to determine (*i*) whether internal loading by entrainment represents a significant part of the lake's epilimnetic P budget and (*ii*) what factors lead to interannual variability in internal loading to the lake.

Methods

Study site

Lake Mendota (3985 ha, 12.7 m mean depth, 25.3 m maximum depth) is a culturally eutrophic lake located in south-central Wisconsin, U.S.A. (43°6′N, 89°24′W). The lake's 600-km^2 watershed is 85% agriculture and 9% urban; the remainder is in forests and wetlands. Since the 1850s, nutrient loadings from the watershed have been high (average annual loading of 0.96 g P·m^{-2}·year^{-1} during the 1970s; Lathrop 1979, 1990), and currently, nutrients deriving from nonpoint agriculture and urban land sources are the dominant external nutrient input to the lake (Lathrop 1979, 1990).

Lake sampling

We measured the following variables to calculate an epilimnetic budget of TP during the summer stratified season for 1992 and 1993 using a mass-balance equation:

$$(1) \quad dSS_p = Ext + Atm + SedR + Entr - Out - Sed + Res$$

The change in P standing stock (dSS_p) is balanced by inputs from external loading (Ext), dry and wet atmospheric deposition (Atm), release of P from epilimnetic sediments (SedR), entrainment (Entr), and outputs from sedimentation (Sed) and outflow (Out). The residual (Res), representing P that is not accounted for, was calculated by difference. We assumed that entrainment was the dominant process by which P is transported from the hypolimnion to the epilimnion (see below). If other processes were operating, they would appear as the residual in the P-budget equation when entrainment is included (eq. 1). All variables were measured directly except for Atm and SedR, which were estimated from past studies conducted on nearby lakes. The stratified season for 1992 lasted from 9 June to 14 September and for 1993, from 21 June to 13 September.

We sampled the lake weekly at the central sampling station located at the deepest part of the lake during the two summers. We collected samples for TP standing stock at six depths every 4 m between 1 and 20 m and also at 22 m. Duplicate sediment traps (three cylinders per trap) were suspended at 22 m and retrieved weekly. Traps were designed with a height to width ratio of 5, and an internal diameter of 5.1 cm (Blomqvist and Kofoed 1981). The three cylinders in each trap were pooled and TP content determined. Daily external loading was calculated from data obtained from continuous flow monitoring stations on two of the five major streams draining into the lake and one of the two main storm sewers connected to the lake (USGS 1994). Total phophous concentrations from storm events and occasionally base flow, were measured from discrete water samples collected using automatic samplers (USGS 1994). The three monitored inflows represent approximately 53% of the TP flowing into the lake (Lathrop 1979). Total phosphorus loading from unmonitored streams was estimated by calculating ratios of TP loading from monitored to unmonitored streams from data for 2 years of complete loading measurements of all the major streams draining into Lake Mendota (Lathrop 1979). Phosphorus loads from the gauged streams and the storm sewer were calculated by direct integration (USGS 1994). Concentrations of P from all inputs and the lake were measured spectrophotometrically by forming phosphomolybdate complexes after persulfate digestion (Strickland and Parsons 1968). Total phosphorus outflow from the Yahara River (the only outflow) was calculated by multiplying daily discharge values (computed from a program that uses the outlet operational records as input and USGS rating equations to calculate total daily discharge) by the average weekly epilimnetic TP concentration measured at the central lake station.

Total chlorophyll and algal biomass was sampled at the central lake station biweekly during both summers from an integrated sample taken from the surface to 8 m. Phytoplankton samples were preserved with glutaraldehyde, filtered, and mounted in a water-soluble methacrylic resin (Crumpton 1987). Biovolumes were calculated based on identification, enumeration, and individual measurements of a subsample of cells or colonies of each algal taxon. Chlorophyll *a* was determined after filtration through glass fibre filters and extraction in 90% acetone, using the trichromatic technique (Kopp and McKee 1979; Lathrop and Carpenter 1992).

Estimating thermocline depth

We measured temperature profiles continuously throughout the summer to examine the dynamics of thermocline migrations during the stratified periods of 1992 and 1993. A permanent buoy with a thermistor chain and datalogger was placed at the central lake station. Thermistors were placed at depths that would best characterize both the epilimnion and thermocline depths throughout the summer. (In 1992, depths were 1, 5, 6, 9, 10, 11, 12, and 16 m.) Based on an analysis of 47 years of temperature data for Lake Mendota, the average

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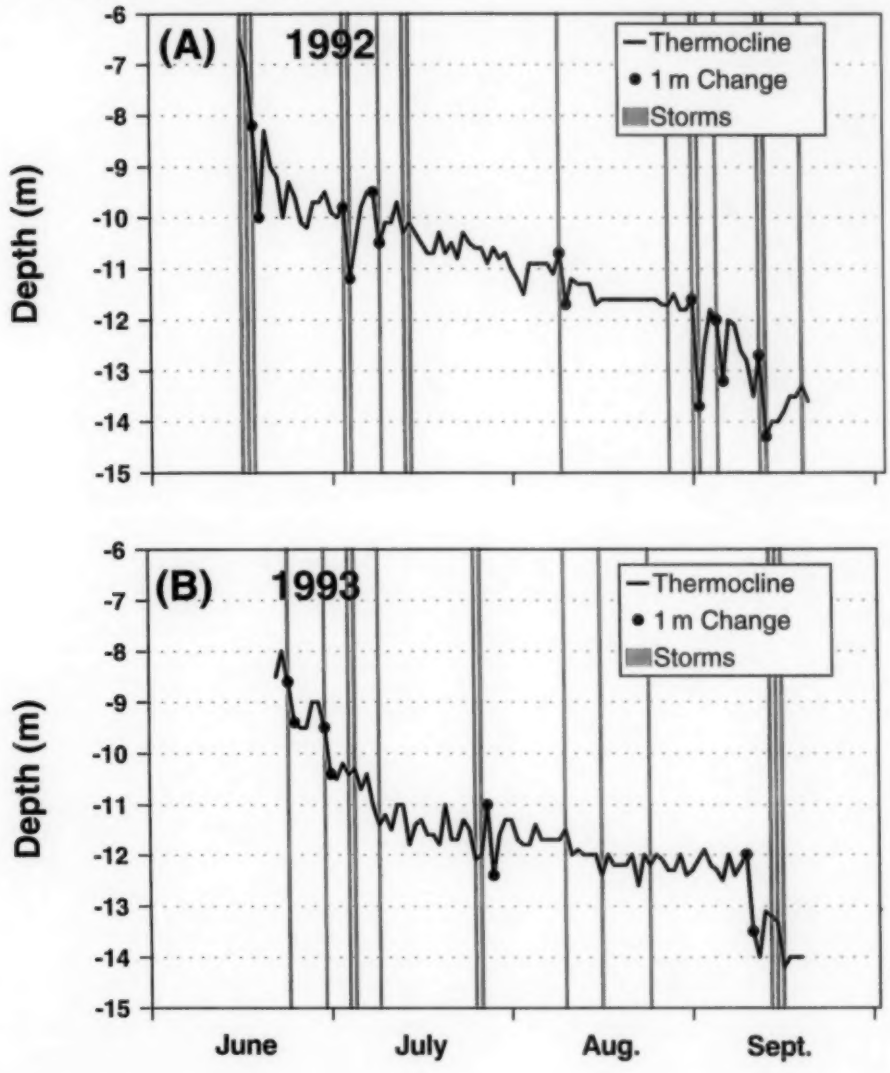
Fig. 1. Daily thermocline depth (solid line) and days of thermocline migrations ≥1 m·day $^{-1}$ (circles) for 1992 (A) and 1993 (B). Days that were classified as storms (see text) are shown as gray vertical bars.



depth of the thermocline from 15 June to 31 August was 10.5 ± 1.7 m (mean ± SD); Robertson 1989). Therefore, we concentrated thermistors between 8 and 12 m. Thermistors placed at 7, 8, and 22 m stopped functioning after a week in the field and could not be replaced. We updated the temperature profiles weekly for the 22 m depth only using temperature profiles taken manually once a week with a portable YSI meter. Temperatures at 22 m changed little from week to week, and this substitution had no effect on thermocline estimates (Soranno 1995). Comparisons of epilimnion and thermocline depths calculated from the thermistor data agreed well with epilimnion and thermocline depths calculated from the temperature profiles taken from weekly YSI meter readings (Soranno 1995). In 1993, thermistors were placed at 1, 5, 6, 8, 9, 10, 11, 12, 14, 16, and 22 m. Thermistors from both years were calibrated in a water bath from 5 to 27.5°C. Temperatures were recorded every 2 min. To remove the effects of internal seiches (period 15.4 h. during July; Stewart 1965), mean daily profiles were computed from each days' 720 recorded profiles and used for all analyses. Also, because the central lake station is located at the node of the primary seiche, temperatures sampled there should be minimally affected by seiches.

Because the thermal structure of medium–large lakes such as Lake Mendota (>500 ha) can be quite dynamic and difficult to characterize (Robertson 1989; Fee et al. 1996), an automated search for thermocline as it is typically defined (the depth at which the maximum temperature change occurs) resulted in erratic and incorrect thermocline and epilimnetic depths (P.A. Soranno, unpublished data). Fee et al. (1996) reached the same conclusion when they consistently were unable to fit a simple exponential curve to temperature versus depth profiles in medium–large lakes in their study of Canadian Shield lakes (surface area ranging from 706 to 34 700 ha). Therefore, we estimated thermocline depth from visual inspection of the temperature profiles.

The actual definition of thermocline depth in larger lakes can also be difficult to characterize. Because of the complex thermal structure in these lakes as well as the presence of secondary or "breeze"

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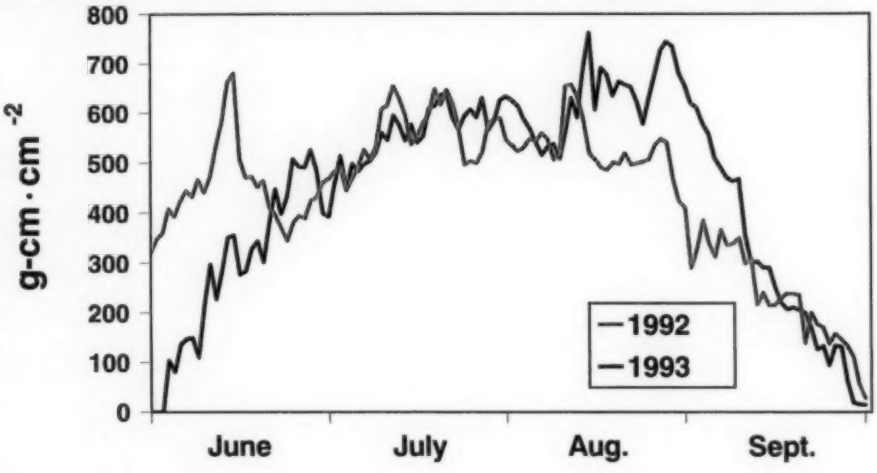
Fig. 2. Schmidt stability (g·cm·cm^{-2}) calculated from daily temperature profiles for 1992 (gray line) and 1993 (black line).



thermoclines that that are sharper than primary thermoclines, thermocline estimates defined as the maximum rate of temperature change per metre, as thermocline depth is commonly defined, resulted in inaccurate estimates of thermocline depth (Soranno 1995). Therefore, we used an alternate approach. We first estimated the temperature of the primary thermocline as the temperature that was midway between the temperatures of the maximum and minimum of the second derivative of the temperature versus depth profile (which are the boundaries for the epilimnion and hypolimnion, respectively). This temperature was determined by visual inspection of temperature profiles interpolated with a cubic spline for each day during the summer and then averaged across the entire summer. The temperature of the primary isotherm in 1992 was 15.8 ± 0.7°C, and in 1993 was 17.5 ± 1.0°C. Thermocline depth was then defined for each day as the depth of this primary thermocline isotherm. The isotherm method has been used in several studies in Lake Mendota, and the temperature of the primary thermocline has been previously identified to be between 17 and 18°C (Stauffer 1974; Robertson 1989). It was necessary to first identify the temperature of the primary thermocline, since there can be some interannual variation in water temperatures. The isotherm method has the advantage of being less sensitive to dynamics of the epilimnion (i.e., complex thermal patterns and breeze thermoclines) and being slightly less dynamic through time so that the large thermocline migrations can be identified more clearly (Soranno 1995).

Phosphorus-entrainment calculations

We calculated daily entrainment of P from the hypolimnion to the epilimnion following deepening of the thermocline. Total P mass transported into the epilimnion was calculated by multiplying the volume of water added to the epilimnion (caused by the deepened thermocline depth) by the average P concentration in the thermocline region (Stauffer 1987). At the daily scale the thermocline is highly variable, so we assumed that only large thermocline movements would entrain significant net amounts of P. Based on visual inspection of the daily thermocline changes, major events always exceeded 1 m in magnitude, which is near the vertical resolution of our temperature record. Therefore, we calculated entrainment using all events in which the thermocline deepened by ≥1 m. Other methods to determine the occurrence of migration events such as identifying thermocline depth immediately before and after storm events (as is commonly done) or using all thermocline migrations resulted in either implausibly low or high estimates of P entrainment, respectively (Soranno 1995). We checked our method for calculating P entrainment by estimating internal loading by difference. Equation 1 was rearranged and a residual (Res) of 0 was assumed:

$$(2) \quad \mathrm{Entr_{diff}} = \mathrm{dSS_P} + \mathrm{Out} + \mathrm{Sed} - \mathrm{Ext} - \mathrm{Atm} - \mathrm{SedR}$$

where $\mathrm{Entr_{diff}}$ is entrainment calculated by difference, and all other terms are as in eq. 1.

Other calculations

Water column stability, S (g cm·cm^{-2}), was calculated from the daily temperature profiles using the Schmidt stability index (Likens 1985):

$$S = A_0^{-1} \Sigma(z - z^*)(\rho_z - \rho^*)A_z \, dz$$

where A_0 is surface area of the lake, A_z is the lake area at depth z, ρ_z is density as calculated from the temperature at depth z, ρ^* is the lake's mean density, and z^* is the depth where the mean density occurs. The summation is taken over all depths (z) at an interval $dz = 1$ m. Schmidt stability is the amount of work required to mix the lake to an isothermal temperature (Likens 1985). It is zero when the lake is isothermal and maximal when the lake is most strongly stratified.

Storm events were estimated from daily precipitation and wind velocity data obtained from the Madison weather station. Storms were defined to be days when daily precipitation was >20 mm, and wind velocity was greater than the summer average wind speed (3.8 m·s^{-1}). Days were also included when wind speed was well above the summer average (>5.0 m·s^{-1}) and where there was at least some precipitation, which usually occurred on days immediately preceding or following storm events as defined above. Although this definition of storms is somewhat arbitrary, our method for calculating P entrainment does not depend on the identification of storms, and we use the storms only to check our identification of migration events.

The only P inputs to the lake that were not directly measured were dry deposition, precipitation, and P release from epilimnetic sediments. Because previous budgets developed for Lake Mendota showed that dry deposition and precipitation were an extremely small portion of the P budget, we did not feel that it was necessary to measure these directly (Lathrop 1979). In developing a P budget for Lake Mendota, Lathrop (1979) determined the best estimate for dry deposition to be based on annual measurements of dry fallout solids sampled by the City of Madison from 1972 to 1977 (630 kg·ha^{-1}·year^{-1}) multiplied by a mean annual total P per unit weight of dry fallout solids measured from urban areas across the country (0.98 mg P·g^{-1} dry solids; Amy et al. 1974). Dry deposition of TP across the summer for Lake Mendota was estimated to be 0.17 mg·m^{-2}·day^{-1}. Total phophorus in precipitation was calculated

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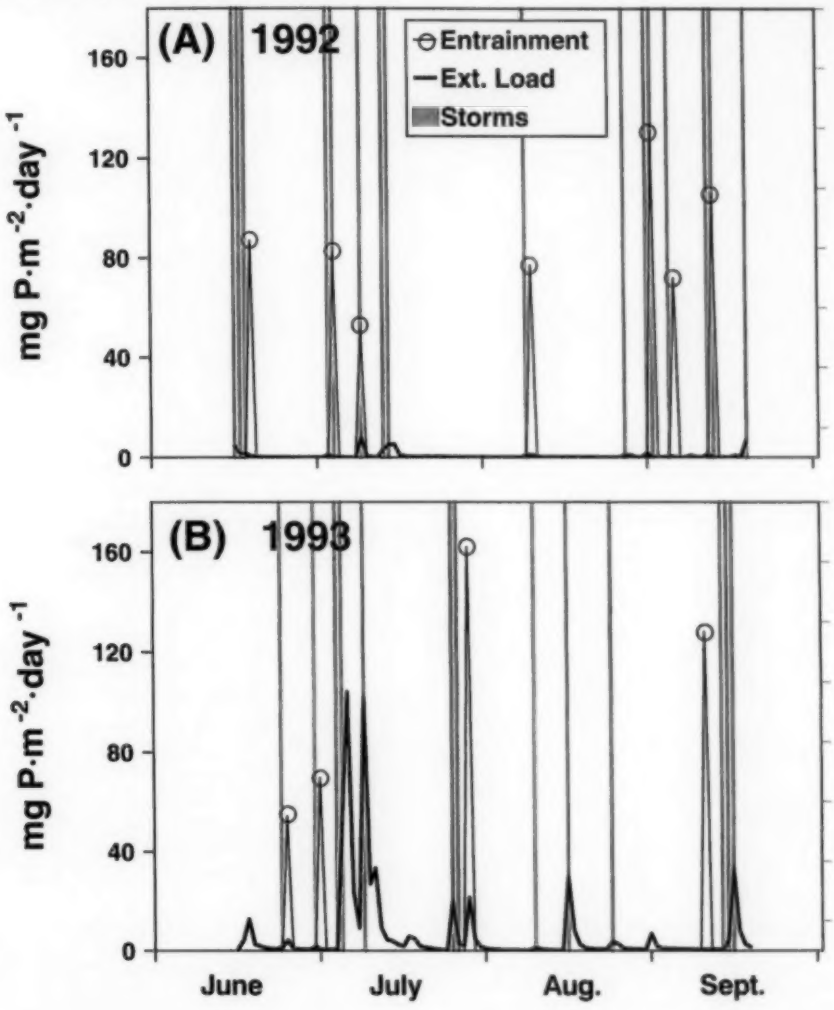
Fig. 3. Daily P entrainment (open circles) and P loads from stream inputs (thick lines; mg P·m^{-2}·day^{-1}) for 1992 (A) and 1993 (B). Days that were classified as storms (see text) are shown as gray vertical bars.



by multiplying daily precipitation volume (obtained from the Madison weather station at the Dane County Truax airport about 7 km northeast of the lake) falling over the lake area by an average TP concentration of precipitation that was measured for nearby Lake Wingra (0.032 mg·L^{-1}; Kleusner 1972).

Although release of P from epilimnetic sediments is a potentially important component in summer P budgets, it is difficult to estimate directly across the entire summer (Stauffer 1987). Based on laboratory and field studies from hardwater Wisconsin lakes, including Lake Mendota (Lee et al. 1977; Gallepp 1979; Holdren and Armstrong 1980; Erickson 1980), Stauffer (1987) determined the average sediment release of P from sediments in contact with the epilimnion in Lake Mendota to range from 1.2 to 3.5 mg P·m^{-2}·day^{-1}. We used the average of this range, 2.4 mg P·m^{-2}·day^{-1} and assumed that the average depth of the epilimnion was 9 m across the summer (average depth from both years) to derive an aerial loading estimate.

Error analysis

In budget studies, errors will depend on the identification of error for each component. Because most of the errors in our budget are poorly known, we have attempted only a rough estimate of the error in our budget by propagating estimated and measured errors through all terms of the above equation (Reckhow and Chapra 1983). Errors were measured directly for sedimentation rates through duplicate sediment traps. Phosphorus profiles measured at the deep lake station in Lake Mendota by Stauffer (1985) were found to underestimate total lake phosphorus by 3%, which is the error term we use. For all other components we assume an arbitrarily chosen coefficient of variation for each variable of 50%.

Results

Weather was substantially different between the two summers. Total precipitation in 1993 during the summer stratified season was the highest of the past 100 years (64 cm in 1993 vs. 40 cm in 1992, an average year), resulting in unusually high runoff in 1993 compared with 1992. But, the number of storms in 1992 was slightly more than in 1993 (16 vs. 13) (Fig. 1). During both years, storms were concentrated in early and late summer,

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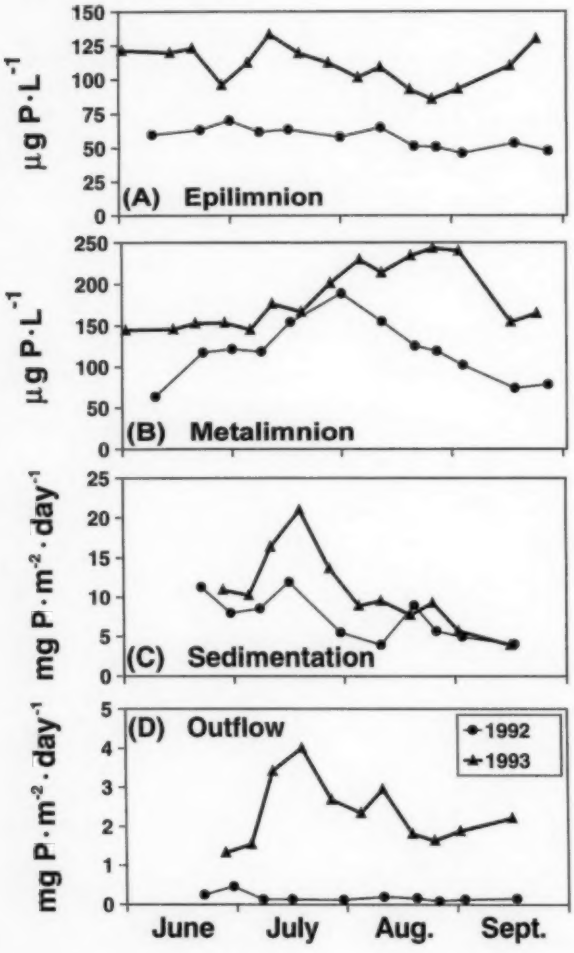
Fig. 4. Daily TP concentrations in the epilimnion (A) and in the metalimnion (depth between 9 and 14 m) (B) in μg $P \cdot L^{-1}$ and measured sedimentation rate (C) and stream outflow (D) in mg $P \cdot m^{-2} \cdot day^{-1}$ for 1992 (circles) and 1993 (triangles). Note the difference in the *Y*-axis scale.



although there were also five storms in midsummer in 1993 compared with only 1 in 1992. Daily thermocline depth was highly variable both years (Fig. 1); and although thermocline migrations ≥1 m·day $^{-1}$ usually coincided with storm events, thermocline migrations did not always occur on the day immediately following the storms. Also, not all storm events resulted in thermocline migrations due in part to differences in water column stability. During midsummer, when the lake is very stable, it is unlikely that storms will result in major thermocline migration. For example, when stability was at a maximum during August 1993 (Fig. 2), three storms had little effect on the thermocline. In contrast, in 1992, stability was especially low in September, and the many storms that occurred at that time resulted in migration events. Also, a major thermocline migration occurred in mid-September when stability was low on a day that we did not classify as a storm. During these 2 days, average wind velocity was 7.2 and 4.9 $m \cdot s^{-1}$ with 0.3 mm precipitation.

Phosphorus entrainment calculated from these thermocline migrations resulted in pulsed nutrient inputs throughout both summers (Fig. 3). There were more entrainment events in 1992 compared with 1993, most likely resulting from a combination of more storms and weaker stratification, especially during late summer. Storms also coincided with stream runoff events (Fig. 3). Both stream and entrainment inputs occurred in relatively discrete pulses with little input during the time period between events.

Total phosphorus concentrations during both years followed typical patterns for eutrophic lakes in that TP built up below the epilimnion during the stratified season (Figs. 4A and 4B). However, there were distinct differences between the 2 years. In 1992, TP concentrations in the metalimnion did not increase beyond early August; but in 1993, metalimnetic TP concentrations continued to increase through the end of September. In addition, TP concentrations in both the epilimnion and metalimnion were substantially higher in 1993 than in 1992, often by a factor of two. Much of the difference in TP standing stock can be attributed to the unusually high external loads to Lake Mendota that occurred in both spring and summer 1993 (Fig. 3; P.A. Soranno, unpublished data). Both sedimentation and outflow of TP increased in response to the elevated external loading and were higher in 1993 than 1992 (Figs. 4C and 4D).

Major P fluxes during the summer differed between the 2 years. During 1992, external loading was lower than the combined loading from epilimnetic sediment release and atmospheric deposition, and all were relatively minor fluxes (Fig. 5A). Internal loading from entrainment was the largest input into the epilimnion. However, during 1993, external loading was about equal to all other inputs (Fig. 5B). Internal loading due to P entrainment was about 10 times higher than external loading during the average runoff year (1992), but was similar to external loading during the high runoff year (1993).

Summer budgets for epilimnetic TP balanced for both years. The residual terms for the mass balance, representing unaccounted-for P, were close to zero for both years (Figs. 5A and 5B). Thus, summer P entrainment calculated by difference using eq. 2 and assuming a residual of zero (0.61 and 0.42 $g \cdot m^{-2}$ for 1992 and 1993, respectively) agreed well with our direct estimate of entrainment (0.60 and 0.42 $g \cdot m^{-2}$ for 1992 and 1993, respectively). Also, both entrainment estimates and sedimentation rates were large relative to the estimated error term for the mass-balance equation, which lends confidence to our budget, since the budget is strongly driven by these two factors. Although the true error for the budget is poorly known, we assumed quite a large error for those parameters with no measured error (CV = 50%), so it is likely that the estimated error is an overestimate.

Discussion

This study confirms the relative importance of internal loading to the summer epilimnetic P budget in a stratified eutrophic lake. During normal rainfall conditions, P entrainment is the dominant source of P to the epilimnion of Lake Mendota. Only during highly unusual weather conditions did external inputs

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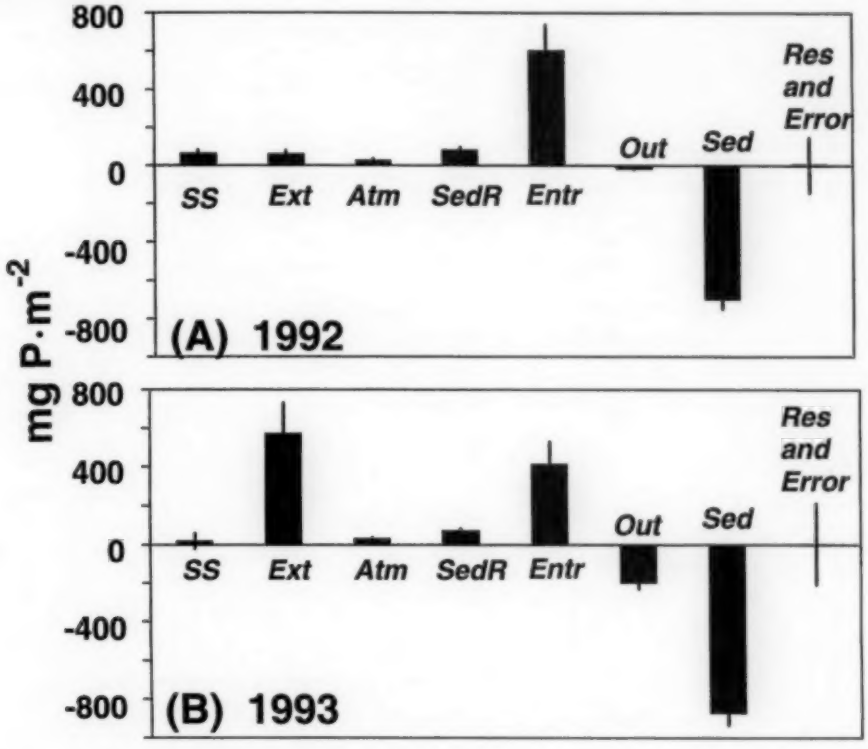
Fig. 5. Epilimnetic TP budgets in mg P·m^{-2} across the summer stratified season for 1992 (A) and 1993 (B). Positive and negative values represent epilimnetic inputs and outputs, respectively. The residual term for both years (Res) is close to zero. The error term propagated for the entire budget is shown as an error bar on the residual term; otherwise, estimated or measured SDs (see text) for each variable are shown on each individual bar. SS is the change in TP standing stock across the summer, Ext is external loading, Atm is atmospheric loading (dry and wet deposition), SedR is release of P from epilimnetic sediments, Out is outflow, Sed is sedimentation from the epilimnion, and Entr is entrainment calculated from temperature profiles.



exceed internal inputs. In addition, we show that P entrainment and sedimentation are the dominant fluxes into and out of the epilimnion, and they appear to balance each other across the summer stratified season as suggested by Stauffer (1987). This is one of the few studies that has measured all of the major fluxes concurrently with estimates of P entrainment and also documented interannual variability in internal P loading in stratified lakes (but see Effler et al. 1986).

Measuring P entrainment in lakes

Average daily temperature profiles showed the thermocline to be a highly dynamic boundary with large daily changes in thermocline depth (Fig. 1). We assumed that the large thermocline migrations (≥1 m·day^{-1}) would capture most of the vertical nutrient flux in a large lake with a dynamic thermal structure. Phosphorus entrainment calculated using such migrations appeared to identify the major migration events and agreed well with the P-entrainment estimate calculated by difference from the mass-balance P budget. Maximum thermocline displacement usually was concurrent with storms (Fig. 1) but did not necessarily occur immediately following a storm. This result suggests that daily sampling of temperature profiles may improve entrainment estimates. If upward vertical fluxes due to less turbulent processes such as eddy diffusion were important, they would have added to the residual term of the mass-balance equation. Although eddy diffusion coefficients can be calculated from temperature profiles and P gradients, the calculation requires many assumptions and usually uses temperature profiles that are more finely spaced vertically (Jassby and Powell 1975). Consequently, we did not attempt to calculate eddy diffusion. It is likely that our estimate of entrainment includes other transport processes but that the flux caused by major storm events is the dominant process by which P is transported from the hypolimnion to the epilimnion in medium–large lakes such as Lake Mendota.

To determine the potential importance of P entrainment of epilimnetic budgets to lakes in general, it would be useful to compare our entrainment results with other lakes. One of the few lakes where a total summer entrainment estimate is presented is Onondaga Lake (12.0 m mean depth and 1170 ha total area), a stratified, eutrophic lake in New York (Effler et al. 1986). Effler et al. (1986) estimated the average entrainment across two summers to be 2.5 mg P·m^{-2}·day^{-1}, which is of similar magnitude to our average estimate of P entrainment for both summers (5.5 mg P·m^{-2}·day^{-1}). However, our estimate differs from a previous entrainment estimate for Lake Mendota (Stauffer 1987), where summer entrainment in 1972 was calculated to be about 0.3 mg P·m^{-2}·day^{-1}. Epilimnetic TP

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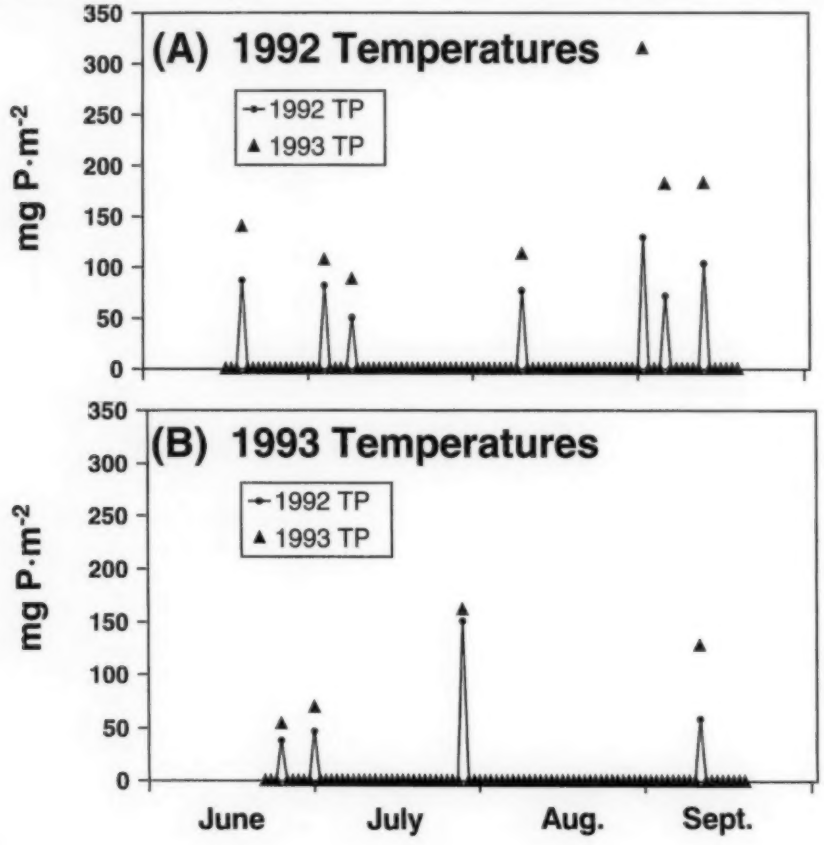
Fig. 6. Phosphorus entrainment calculated for 1992 temperature data with TP concentration data from 1992 (circles) and 1993 (triangles) (A) and P entrainment calculated for 1993 temperature data with TP concentration data from 1993 (triangles) (A).



concentrations in 1972 were similar to those of 1992 (Stauffer 1987), so it is unlikely that there were major differences in nutrient dynamics in the lake during 1972 that would explain this large difference between the two entrainment estimates. Although we used similar methods to calculate entrainment, Stauffer (1987) used a sampling frequency that was roughly weekly, compared with our daily sampling. Entrainment may be underestimated using weekly sampling, because major migration events occur at finer temporal scales and because thermocline depth returns to prestorm levels soon after the storm passes (Fig. 1).

Phosporus entrainment in Lake Mendota

Estimates of P entrainment to the epilimnion in Lake Mendota during both summers were large relative to other terms of the P budget. Even though external loading to Lake Mendota was high because of increased precipitation and flooding throughout the summer of 1993, these conditions did not lead to large thermocline migrations as we had expected from the high number and intensity of storms. In studying the effect of wind stress on thermal structure in Lake Mendota, Stauffer (1992) found that the highest wind velocity events did not necessarily lead to the highest thermocline migrations, in part because of different initial water column stabilities at the time of the storm. External and internal loading are coupled with weather and thermal stratification in complex ways.

Entrainment potential

The potential for P to be entrained into the epilimnion should depend on two things: (*i*) the mixing potential and (*ii*) the nutrient loading potential. First, mixing potential will depend on water column stability coupled with the number and strength of storm fronts that cause the thermocline to migrate (Stauffer and Lee 1973; Effler et al. 1986). Like Effler et al. (1986), we found that entrainment events were concentrated during early and late summer when stratification is weaker and the lake more subject to mixing, although some events did occur midsummer (Fig. 3). Although there were more storms that led to thermocline migrations of 1 m or more in 1992 than in 1993 (Fig. 1), this was most likely due to a combination of a greater number of major storms and weaker stratification late in the summer. Thus, total P entrainment was higher in 1992. The other factor determining P entrainment is the nutrient loading potential. In 1993, P concentrations in the metalimnion were higher than in 1992, increasing the potential internal load (Fig. 6). To compare the roles of these two mechanisms, we recalculated entrainment for each year using each year's temperature data with TP data from the other year (Fig. 6). In

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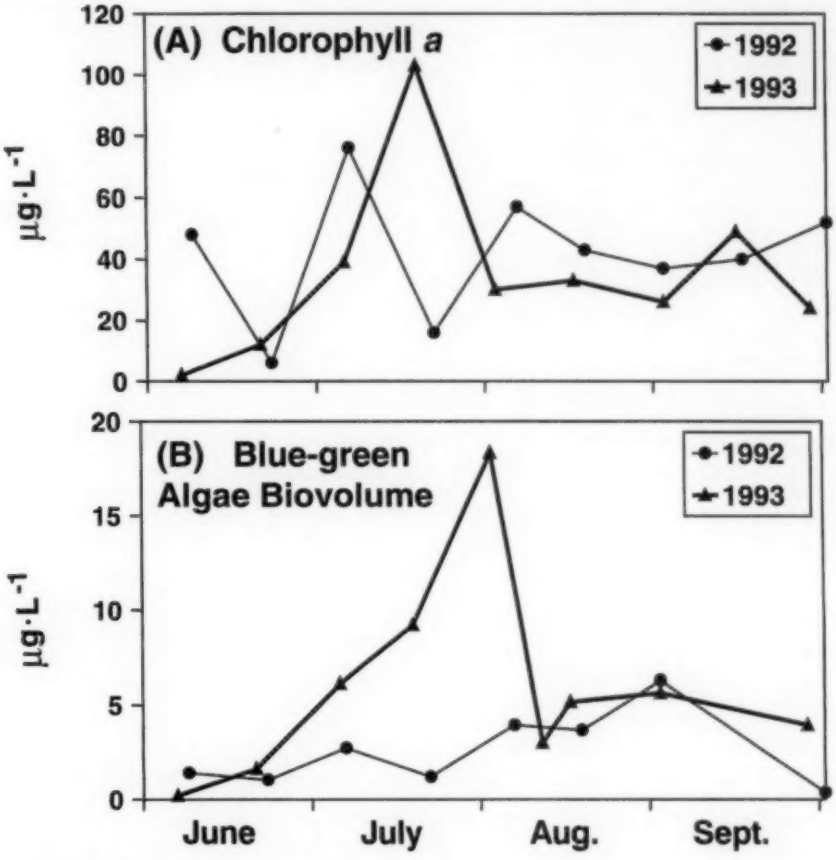
Fig. 7. Chlorophyll *a* concentration in $\mu g \cdot L^{-1}$ (A) and blue-green algae biovolume in $\mu L \cdot L^{-1}$ (B) during summer for 1992 (circles) and 1993 (triangles).



1992, total summer entrainment calculated using TP data from 1993 was about 11.5 mg $P \cdot m^{-2} \cdot day^{-1}$, almost two times higher than that calculated with 1992 TP data (6.1 mg $P \cdot m^{-2} \cdot day^{-1}$). In contrast, total summer entrainment calculated for 1993 using TP data from 1992 was about 3.5 mg $P \cdot m^{-2} \cdot day^{-1}$, almost 1.5 times lower than that calculated using TP data from 1993 (4.9 mg $P \cdot m^{-2} \cdot day^{-1}$). These values may bracket high and low entrainment values possible in Lake Mendota. Daily entrainment calculated using TP data from 1993 was always higher than that calculated using TP data from 1992. There also appears to be a seasonal difference in this contrast. The largest discrepancies between the two entrainment calculations during both years were in late summer when metalimnetic TP concentrations were maximum in 1993, but were declining in 1992 (Fig. 4B).

Consideration of internal loads from entrainment may help explain some of the observed interannual variability in epilimnetic TP dynamics in stratified lakes such as Lake Mendota. For example, when spring and summer external loads are low, metalimnetic TP will be relatively low. In addition, if summer conditions are such that stratification is stable with few storm fronts, P entrainment should be relatively low. Such conditions occurred in Lake Mendota in 1988 during a regional drought and very warm summer resulting in a very stable water column and unusually low summer TP concentrations (Lathrop 1992). Conversely, with high winter and spring TP loads, low stability, and frequent storm fronts, internal loading can be large. This extreme situation was simulated by using TP data from 1993 and stratification data from 1992 (Fig. 6) and may represent the upper bound on P entrainment currently in Lake Mendota, although the full range of possibilities could not be addressed without data from a wider range of weather conditions.

Nutrient loading to the epilimnion

Our assessment of P loads to the epilimnion during these 2 years would be quite different had we not accounted for internal loading. External loads to the epilimnion during summer 1993 (7.0 mg $P \cdot m^{-2} \cdot day^{-1}$) were much higher than in 1992 (0.9 mg $P \cdot m^{-2} \cdot day^{-1}$). However, total loads (internal + external) during the 2 years were more similar: 12.5 mg $P \cdot m^{-2} \cdot day^{-1}$ in 1993 and 7.8 mg $P \cdot m^{-2} \cdot day^{-1}$ in 1992. In fact, our data suggests that the total range in internal loading may be smaller than the observed range in external loads, which is important because internal loading represents from 38% (during a high runoff year) to 78% (during an average runoff year) of the total loads to the epilimnion in Lake Mendota. Consequently, internal P loading may act to buffer large interannual fluctuations

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in external inputs to the lake. Adding factors related to internal loading from P entrainment to summer nutrient models may help predictions of lake response to changes in external nutrient inputs.

Ultimately, the ecological significance of these interannual fluctuations in internal loading will depend on the fate of P in the water column and its effect on the algal community. Algae in Lake Mendota appeared to respond to the observed yearly differences in total P loads. Although chlorophyll concentrations during the 2 years were similar (Fig. 7A), during both summers, blue-green algae dominated the algal biomass and represented on average 85% and 96% of the total algal biomass in 1992 and 1993 respectively. In addition, blue-green algal biomass was above nuisance levels (2 $\mu L \cdot L^{-1}$; Lathrop and Carpenter 1992) most of the study period and was, on average, about two times higher in 1993 than 1992 (Fig. 7B).

Another factor that may determine the ecological relevance of internal loading from P entrainment is the relative proportion of internal and external loads to the total loads. Phosphorus entrainment contributed 78% of the total loading in 1992 but only 38% in 1993. Internal loading has been shown to have higher levels of biologically available P than external sources (Nürnberg 1988). In fact, in a July and August sample taken from the metalimnion in 1992, 89 and 78% of the TP, respectively (R.C. Lathrop, unpublished data) was dissolved reactive phosphorus (DRP), a form of P that is available for algal uptake. These high levels of DRP from internal sources are important because typically in Lake Mendota, epilimnetic DRP concentrations are below analytical detection (Lathrop and Carpenter 1992) and hence most likely growth limiting to algae (Brownlee and Murphy 1983; Prepas 1983). In summers where entrainment events are large, more P is supplied to the epilimnion leading to higher algal biomass. Only when runoff is unusually high is DRP above analytical detection, as occurred in 1993, and probably not growth limiting to algae. In addition, dissolved N:P ratios of both sources may differ and influence the response of blue-green algae in particular. The relative importance of internal versus external sources of nutrients in explaining interannual variability in algal dynamics remains an important question.

Within lakes such as Lake Mendota, variation in internal loading from P entrainment can be explained in part by external loads, which determine nutrient gradients, and seasonal weather patterns (i.e., storm frequency), which influence stratification and thermocline dynamics. The highest internal loads should occur when metalimnetic P concentrations are high because of high spring and summer external loads and stratification is weak and variable because of storm fronts during the summer. The lowest entrainment should occur when external loads are low and the water column is stable with few storm fronts.

Acknowledgments

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Allometry of diet selectivity in juvenile Atlantic salmon (*Salmo salar*)[1]

Ernest R. Keeley and James W.A. Grant

Abstract: Juvenile salmonids in streams typically feed on larger invertebrates than the average size available in the drift. Our objective was to describe the allometry of this size-selective foraging in juvenile Atlantic salmon, *Salmo salar* of Catamaran Brook, New Brunswick. We compared paired samples of the stomach contents of 46 salmon (age 0+ to 2+; fork length 2.9–14.5 cm) with drift samples collected from their feeding territories. Juvenile salmon fed opportunistically on all major types of invertebrates in the drift, except for water mites (Hydracarina). However, newly emerged salmon fed on smaller prey than the average available in the drift, primarily chironomid larvae, whereas salmon larger than 4.6 cm fed on larger prey than average, primarily dipteran adults and pupae. Larger salmon ate larger prey. Minimum prey length in stomachs was well predicted by gill raker spacing, but mean prey width was only one third of the optimal size and maximum prey width was much less than mouth width. The allometry of prey size appeared to be related primarily to an increase in size-selective foraging with increasing body size, rather than to morphological constraints. Juvenile Atlantic salmon in our study ate smaller prey than similar-sized salmonids in other studies.

Résumé : Les salmonidés juvéniles dans les cours d'eau se nourrissent typiquement d'invertébrés de taille plus grande que la taille moyenne disponible dans la dérive. Notre objectif était de décrire cette allométrie dans ce broutage sélectif en fonction de la taille chez le saumon atlantique juvénile (*Salmo salar*) du ruisseau Catamaran, au Nouveau-Brunswick. Nous avons comparé des échantillons variés de contenu stomacal de 46 saumons (âge 0+ à 2+; longueur à la fourche 2,9–14,5 cm) avec des échantillons de dérive prélevés dans leur territoire d'alimentation. Le saumon juvénile s'alimente de manière opportuniste de tous les principaux types d'invertébrés présents dans la dérive, à l'exception des hydrachnes (*Hydracarina*). Toutefois, les saumons nouvellement émergés s'alimentaient de proies dont la taille était plus petite que la taille moyenne des organismes disponibles dans la dérive, principalement des larves de chironomidés, tandis que les saumons de taille supérieure à 4,6 cm prélevaient des proies de taille plus grande que la moyenne, principalement des adultes et des pupes de diptères. Les saumons de plus grande taille consommaient des proies de plus grande taille. La longueur minimale des proies dans les estomacs pouvait être prédite efficacement par l'espacement du peigne branchial, mais la largeur moyenne des proies n'était que le tiers de la taille optimale et la largeur maximale des proies était de beaucoup inférieure à la largeur de la bouche. L'allométrie touchant la taille des proies semble être liée principalement à une augmentation du broutage sélectif en fonction de la taille, plutôt qu'à des contraintes morphologiques. Les saumons atlantiques juvéniles de notre étude ont consommé des proies plus petites que celles qui étaient consommées par des salmonidés de taille semblable dans d'autres études.
[Traduit par la Rédaction]

Introduction

Stream-dwelling salmonids feed primarily on drift, invertebrates carried by water currents (McNicol et al. 1985; Keeley and Grant 1995; but see Tippets and Moyle 1978). Diet selectivity in salmonid fishes has often been studied by comparing the invertebrates found in stomach samples with those found in drift samples. Such studies have shown that stream-dwelling salmonids feed opportunistically on a wide variety of prey types; there is usually a positive correlation between the proportions of invertebrates in the diet and in the drift (Allen 1941; Allan 1981, 1995; Angradi and Griffith 1990). However, residual variation about this relationship is usually related to invertebrate size such that large invertebrates are usually over-represented in the diet, whereas small invertebrates are under-represented (Allan 1981).

The diet of fishes often change continuously with body size (Werner and Gilliam 1984). The few allometric studies of diet in stream-dwelling salmonids have documented an increase in prey size with body size (Fahy 1980; Rose 1986), perhaps because they become increasingly size selective as they grow larger (Newman and Waters 1984; Grant and Noakes 1986). However, no study has yet described how diet selectivity changes continuously with changes in body size.

Functional reasons for changes in diet and (or) selectivity with body size have been linked to morphological constraints and to feeding energetics. Minimum prey length is thought to be limited by gill raker spacing (Wańkowski 1979; Dunbrack and Dill 1983), whereas maximum prey width is thought to be limited by mouth width (Wańkowski 1979). Both gill raker spacing and mouth size increase with body size (Wańkowski 1979). Within the range of prey sizes set by morphological constraints, fishes are expected to select prey to maximize their energetic gain per unit time (Werner and Hall 1974). Because

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E.R. Keeley[2] and J.W.A. Grant. Department of Biology, Concordia University, 1455 de Maisonneuve Blvd. West, Montreal, QUE H3G 1M8, Canada.

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[2] Author to whom all correspondence should be sent at the following address: Department of Zoology, The University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada. e-mail: keeley@zoology.ubc.ca

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Table 1. Environmental correlates (Pearson product–moment correlation) of the number (number·m^{-2}·h^{-1}) and mean width (mm) of invertebrates captured in 46 drift samples.

Environmental variable[a]	Mean	Range	Number		Width	
			r	Partial r[b]	r	Partial r[b]
Current velocity (cm·s^{-1})	21.0	1–45	0.52**	0.65**	0.045	0.13
Water depth (cm)	31.5	4.0–67.3	0.30*	0.48*	–0.14	–0.092
Substrate size[c]	3.1	2.0–4.1	0.29*	0.057	–0.067	–0.041
Day of the year	213.5	167–244	–0.33*	–0.72**	–0.12	–0.071
Fish length (cm)[d]	6.4	2.9–14.5	0.24	–0.096	–0.092	–0.002

Note: *$P \leq 0.05$; **$P \leq 0.01$.

[a]Correlations are based on $\log_{10}$-transformed data for current velocity, water depth, prey numbers, and prey width.

[b]Correlation coefficient of an environmental variable versus the residual variation from a multiple correlation including all other environmental variables.

[c]Categorized according to Bain et al. (1985).

[d]Length of fish occupying the territory in which the drift sample was taken.

Table 2. Environmental correlates (Pearson product–moment correlation) of the number and width (mm) of prey in the stomachs of 46 juvenile Atlantic salmon.

Environmental variable[a]	Number		Width	
	r	Partial r	r	Partial r
Current velocity	–0.097	0.04	0.32*	–0.041
Water depth	0.26	0.29	0.58**	0.18
Substrate size	–0.13	–0.14	0.52**	0.12
Day of the year	0.31*	0.20	0.21	–0.16
Fish length	–0.13	–0.22	0.78**	0.58**

Note: *$P \leq 0.05$; **$P \leq 0.01$

[a]Units and footnotes are as in Table 1.

Table 3. $\log_{10}$ prey size (mm) versus $\log_{10}$ fork length (cm) for 46 juvenile Atlantic salmon.

Dependent variable	Slope	Y intercept	r^2	P value
Prey width				
Mean	1.042	–1.11	0.61	<0.001
Maximum	1.16	–0.77	0.60	<0.001
Minimum	0.79	–1.35	0.43	<0.001
Prey length				
Mean	0.64	–0.068	0.60	<0.001
Maximum	0.85	0.039	0.60	<0.001
Minimum	0.37	–0.25	0.08	=0.065

larger fishes can handle prey of a given size more quickly than smaller fishes, optimal prey size is expected to increase with body size (Wańkowski 1979; Bannon and Ringler 1986).

Although the diet of juvenile Atlantic salmon has been extensively studied (Allen 1941; Egglishaw 1967; Lillehammer 1973; Wańkowski 1979; Williams 1981; Thonney and Gibson 1989; Cunjak 1992), no study has yet described the allometry of diet for a wide range of body sizes. We collected drift samples and stomach samples of juvenile salmon, 2.9–14.5 cm long, to describe the allometry of diet and diet selectivity. In addition, we determined whether changes in diet with body size could be best explained by morphological constraints or by models of optimal prey size.

Materials and methods

Study site and species

Data were collected from 15 June to 1 September 1992 at Catamaran Brook, a third-order tributary of the Little Southwest Miramichi River (46°52.7′N, 66°06.0′W) in central New Brunswick. We captured fish in the lower 2 km of the brook and in a 1-km section, 5 km upstream from the mouth of the brook. Adult Atlantic salmon return to spawn in the stream from mid-October to mid-November each fall. Young-of-the-year salmon emerge by mid-June at about 2.6 cm in length (Randall 1982) and remain in the stream until they are 2–3 years of age or 12 cm in length (Cunjak et al. 1993). A more detailed description of the stream and its biota can be found in Cunjak et al. (1990, 1993).

We collected 46 juvenile Atlantic salmon that we had individually observed as part of a study on territoriality (see Keeley and Grant 1995). The fish were captured by angling or by using two aquarium dipnets immediately following our observations, all of which were between 09:00 and 16:00. After observing an individual fish, we weighed (±0.05 g), measured fork length to the nearest 0.5 mm, and preserved it in 10% buffered formalin. We collected 26, 12, and 8 salmon of age 0+ (fork length 2.9–5.1 cm), 1+ (6.3–9.5 cm), and 2+ (10.3–14.5 cm), respectively (see Keeley and Grant 1995).

We later used a dissecting microscope, fitted with an ocular micrometer, to identify, count, and measure intact prey items in the esophogus and stomach. Prey items were identified to the order or family level using keys in Merritt and Cummins (1984) or Pennak (1978). Maximum prey length and width (±0.01 mm) were measured for each prey item.

Environmental variables

Within 1.5 h of capturing an individual fish, we sampled food availability on its territory. Because almost 99% of foraging attempts are directed towards items drifting within the water column (Keeley and Grant 1995), we sampled food with a drift net (15.2 cm wide × 23 cm high × 100 cm long, mesh size 300 μm) for 1 h. The mouth of the drift net was constructed with a rectangular metal collar, that was 12.5 cm deep, to maintain a laminar flow at the mouth of the net and to prevent back-washing (see Field-Dodgson 1985).

Each drift sample was transferred to a holding jar for sorting that evening or the next morning. Inedible material, such as decomposing leaves and insect exuviae, was removed from the sample before preservation in 10% buffered formalin. Animals in the drift were identified and measured in the same manner as described for stomach contents. We then dried each sample in an oven at 50°C for 24 h before moving them to a desiccator for an additional 24 h. The dry mass of each sample was determined at 24 h intervals until a stable mass (±0.0002 g) was obtained.

Using a Marsh–McBirney meter, we measured current velocity at each fish's primary holding station (see Keeley and Grant 1995), the

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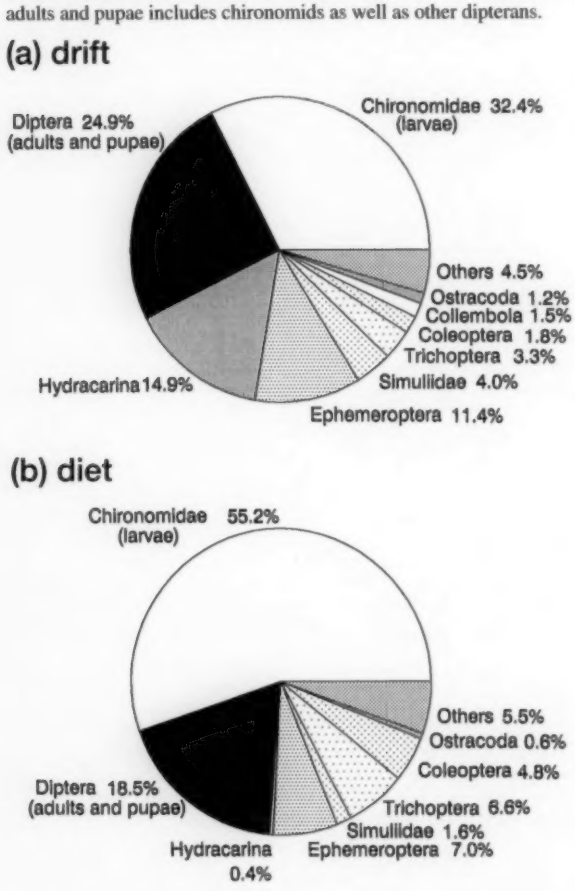
Fig. 1. Taxonomic composition (%) of invertebrates in (*a*) drift samples (5210 organisms in 46 samples) and (*b*) stomach samples (1287 organisms in 46 stomachs) of juvenile Atlantic salmon. Chironomidae refers to larval chironomids only, whereas Dipteran adults and pupae includes chironomids as well as other dipterans.



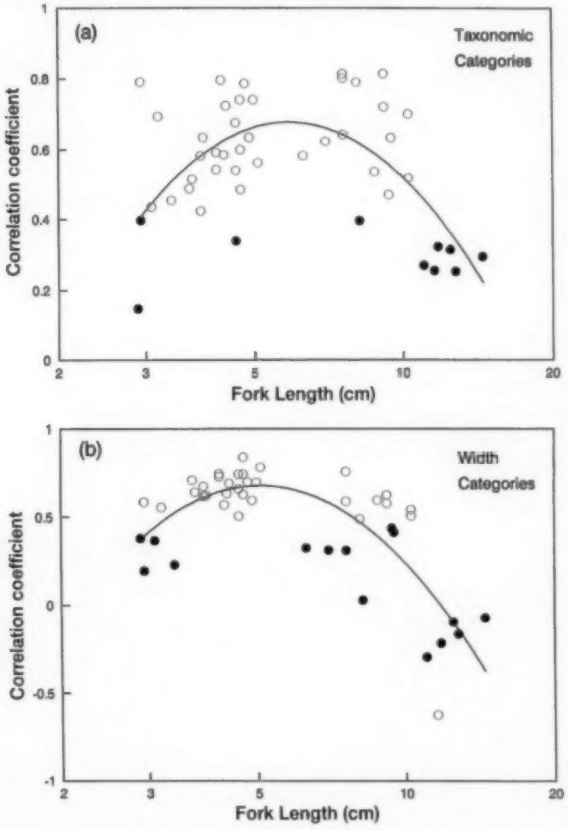
location where the fish normally held its snout. Substrate size was measured by placing two metre sticks parallel and perpendicular to the current, centred on the territory of each fish. We estimated the dominant substrate type (1: < 2 mm; 2: 2–16 mm; 3: 17–64 mm; 4: 65–256 mm; 5: >256 mm in diameter) at 10-cm intervals (after Bain et al. 1985). The intervals were averaged to estimate substrate size for each territory.

Analyses

We used Spearman's rank correlation when data did not meet the assumptions required for parametric analyses. Otherwise, simple or partial parametric correlations were Pearson product–moment correlations. Predictive regressions were calculated using the ordinary least squares technique with log10 transformed data for body size measurements because allometric relationships are usually lognormally distributed (Harvey 1982). We also used $\log_{10}$-transformed data for current velocity and depth measurements because these variables also appear to be lognormally distributed. For some comparisons, we replotted arithmetic relationships from the literature on a logarithmic scale.

We estimated two morphological constraints that might limit the size range of prey that salmon can eat: gill raker spacing (mm) = 0.115 × fork length (cm), and mouth width (mm) = 1.05 × fork length (cm); both allometric relationships were measured by Wańkowski (1979). In addition, we estimated optimal prey size in two ways. First, we used Wańkowski's (1979) estimate of the prey width that maximized growth rate of Atlantic salmon: prey width (mm) = 0.26 × fork length (cm). Second, we used Bannon and Ringler's (1986) estimates of optimal prey width (0.32 × mouth width) and length (1.2 × mouth width) that maximized energy gained per handling time for brown trout (*Salmo trutta*). To compare optimal prey size predicted by Bannon and Ringler's model as a function of fish length, we used Wańkowski's relationship for mouth width versus fork length to translate Bannon and Ringler's (1986) prediction as a function of fork length. Over our size range of fish, we calculated an optimum prey width (mm) of 0.31 × fork length (cm) and an optimum prey length (mm) of 1.15 × fork length (cm).

Fig. 2. Spearman's rank correlation of percent composition of invertebrates in drift samples versus stomach contents in relation to body length for each of 46 juvenile Atlantic salmon by (*a*) taxonomic categories (equation of curve is $Y = 4.57 \log_{10} X - 2.98 \log_{10} X^2 - 2.74$, $r^2 = 0.37$, $P < 0.001$; where Y is the rank correlation coefficient and X is fish length) or (*b*) 0.1-mm width categories (equation of curve is $Y = 7.19 \log_{10} X - 5.09 \log_{10} X^2 - 1.86$, $r^2 = 0.61$, $P < 0.001$; X and Y are as in (*a*)). Open circles represent rank correlations that are significant ($P < 0.05$) whereas closed circles represent rank correlations that are not significant ($P > 0.05$).



Results

Environmental correlates of invertebrate drift

A total of 5210 organisms were captured in the 46 1-h drift

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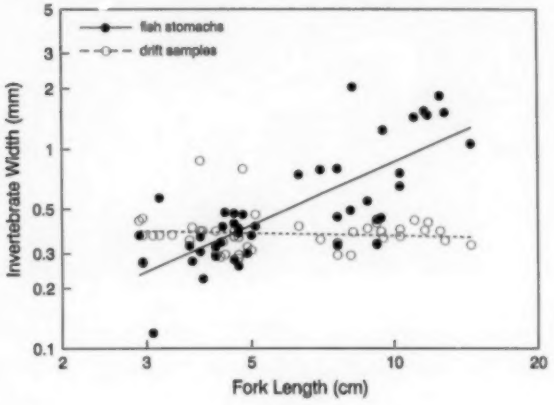
Fig. 3. Relationship between average invertebrate width in stomach samples (solid circles and line; equation in Table 3) and in drift samples within the territory of that fish (open circles and broken line; $\log_{10}$ width (mm) = –0.042 $\log_{10}$ fork length (cm) – 0.39, r^2 = 0.008, P = 0.54) versus fork length for 46 juvenile Atlantic salmon.



samples. The number of organisms in drift samples (108.5 ± 85.4 (mean ± SD), range 5–342) was positively correlated with increasing current velocity, water depth, and substrate size and negatively correlated with day of the year (Table 1). A partial correlation analysis indicated that drift abundance increased with increasing current velocity and water depth but decreased with day of the year (Table 1). Substrate size and the size of the juvenile salmon occupying the site sampled were not significant predictors of drift abundance after statistically controlling for the effect of other variables. The multiple regression model that best describes drift abundance is as follows: number of invertebrates ($\log_{10}$ number·m^{-2}·h^{-1}) = 4.43 + 0.59 $\log_{10}$ current velocity (cm·s^{-1}) + 0.58 $\log_{10}$ water depth (cm) – 0.012 day of year (r^2 = 0.66, P < 0.001). The results of the partial correlation analysis were very similar when we used dry mass of drift samples as the dependent variable. The multiple regression that best describes dry mass of drift samples is as follows: dry mass ($\log_{10}$ mg·m^{-2}·h^{-1}) = 3.67 + 0.56 $\log_{10}$ current velocity (cm·s^{-1}) + 0.41 $\log_{10}$ water depth (cm) – 0.014 day of the year (r^2 = 0.63, P < 0.001).

Despite a wide size range of invertebrates in the drift (width 0.39 ± 0.11 mm, range 0.04–5.58 mm), none of the environmental variables was significantly correlated to the average width of prey in the drift samples (Table 1).

Environmental correlates of diet

A total of 1287 intact organisms were identified in the 46 stomach samples. The number of prey items found in the stomachs of individual fish (27.9 ± 22.9, range 3–134) was weakly related to day of the year and not significantly related to any environmental variable in a partial correlation analysis (Table 2).

The average width of prey items in stomach samples was positively correlated with current velocity, water depth, substrate size, and fish length (Table 2). However, larger fish use deeper, faster areas of the stream that have substrates of larger size (Keeley and Grant 1995). Hence, in a partial correlation analysis, only fish size was significantly related to mean prey size in stomachs (Table 2).

The average, maximum, and minimum size of prey in stomach samples increased with fish size (Table 3). All measures of prey size increased significantly with fish size except for minimum prey length (Table 3). Hence, we used prey width as our measure of prey size in subsequent analyses.

Comparison of drift versus diet

Invertebrates found in both the drift and stomach samples were dominated by a few taxa (Figs. 1*a* and 1*b*). Larval chironomids and dipteran pupae and adults were the two most abundant taxa in both sample types. The most notable difference between the two sets of samples was the proportion of water mites (Hydracarina); they composed 14.9% of the drift samples but only 0.4% of the stomach samples.

The proportion of prey types found in the combined sample of 46 stomachs was positively correlated with their availability in the combined drift samples, whether we divided the invertebrates into taxonomic categories (r_s = 0.56, n = 31, P <0.01) or into 0.1-mm width categories (r_s = 0.70, n = 20, P <0.01). However, because rare taxa may greatly influence the strength of such a relationship (Angradi and Griffith 1990), we repeated the analysis including only those taxa that composed at least 1% of the diet or the drift. Once again, the proportion of prey in the diet was positively correlated with the proportion of prey in the drift (taxonomic categories: r_s = 0.64, n = 17, P < 0.01; width categories: r_s = 0.78, n = 16, P < 0.01).

A comparison of the diet versus the drift for each fish suggests that diet selectivity was influenced by body size of the fish. The correlation between the proportion of invertebrates by taxa in the drift sample and the stomach sample was positive in all 46 cases and significantly positive in 36 cases (Fig. 2*a*). The strength of this positive correlation initially increased with increasing fish body size and then decreased for the largest fish (Fig. 2*a*). These changes in selectivity with body size were even stronger when the data were analyzed by body width category of invertebrate. There was a significant positive correlation between the proportion of invertebrates in the drift and diet in 30 of the 46 fish (Fig. 2*b*). Once again, the strength of this positive correlation initially increased with increasing body size and then decreased; the correlation was negative for fish larger than 10 cm in length and significantly negative for one fish. In summary, the smallest age 0+ fish and the largest age 2+ fish appeared to be the most selective foragers with respect to taxonomic category or size of invertebrate in their stomachs.

Overall, fish ate larger invertebrates than were found in drift samples (paired t = 3.17, n = 46, P = 0.003). However, the degree of size selectivity was a function of the size of the fish. Small fish preyed selectively on small invertebrates whereas large fish preyed selectively on large invertebrates (Fig. 3). The difference between mean invertebrate width in the diet and drift increased with increasing body size (width difference (mm) = 0.113 fork length (cm) – 0.516, n = 46, r^2 = 0.57, P <0.001). The switch from negative size selectivity to positive size selectivity occurred at a body length of 4.6 cm.

The degree of size selectivity was also related to prey type. Overall, the size of chironomid larvae in the diet and the drift did not differ significantly (paired t = –0.32, n = 41, P = 0.75; Fig. 4*a*). However, the proportion of chironomids in the diet

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Fig. 4. Frequency distributions of width of invertebrates in drift and stomach samples for (*a*) chironomid larvae, (*b*) dipteran pupae and adults, (*c*) ephemeropteran larvae, and (*d*) trichopteran larvae. Open bars represent frequency of invertebrates in drift samples and solid bars represent frequency in stomach samples.

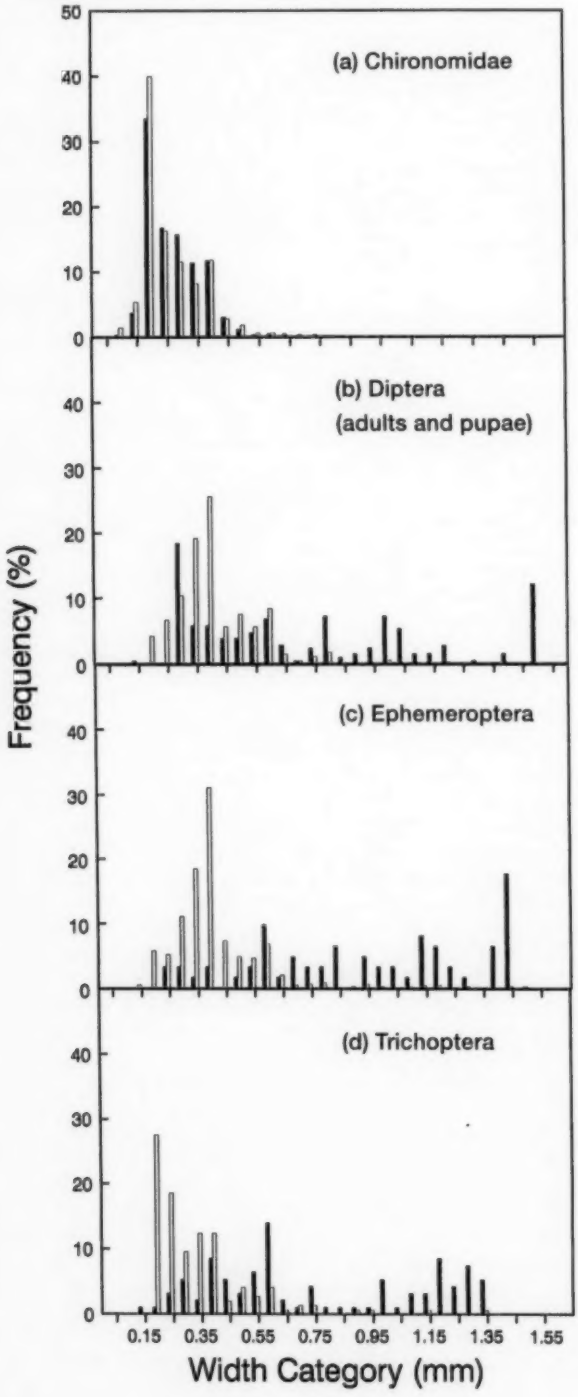
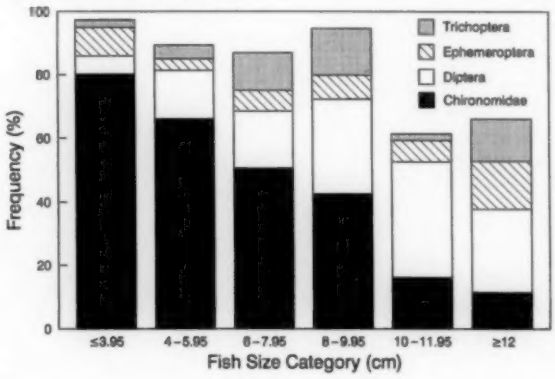


Fig. 5. The average frequency of the four most abundant taxonomic groups of invertebrates found in the stomachs of juvenile Atlantic salmon of six size categories.



decreased with increasing body size of fish ($r = -0.75$, $P < 0.0001$; Fig. 5), perhaps because chironomids were relatively small prey (Fig. 4). In contrast, the size of items in stomachs were larger than those in the drift for dipteran adults and pupae (paired $t = 4.07$, $n = 41$, $P = 0.0002$; Fig. 4*b*), ephemeropterans (paired $t = 6.14$, $n = 25$, $P = 0.0001$; Fig. 4*c*) and trichopterans (paired $t = 5.45$, $n = 19$, $P = 0.0001$; Fig. 4*d*). The proportion of dipterans in the diet increased with body size ($r = 0.52$, $P = 0.0002$), whereas the proportion of ephemeropterans ($r = 0.16$, $P = 0.29$) and trichopterans ($r = 0.26$, $P = 0.08$) in the diet did not change significantly with body size (Fig. 5).

Constraints on size selectivity

The range of prey size in stomachs increased with increasing body size (Figs. 6*a* and 6*b*), such that the difference between maximum and minimum prey size increased with fish length for both prey width ($r = 0.76$, $n = 46$, $P < 0.001$) or prey length ($r = 0.68$, $n = 46$, $P < 0.001$). Age 0+ salmon (i.e., ≤5.1 cm) ate most sizes of invertebrates that were available in the drift. They did not eat the largest or the smallest 5% of invertebrates. Fish longer than 10 cm ate the very largest invertebrates that were available but avoided small prey, primarily small chironomid larvae.

The optimal prey width predicted by Wańkowski (1979) and by Bannon and Ringler (1986) were about three times larger than the mean prey size in stomachs (Fig. 6*a*). The predicted optimal prey widths were similar to the observed maximum prey widths in stomach samples. The invertebrates available in the drift were much smaller than the mouth width of Atlantic salmon (Fig. 6*a*). Minimum prey length corresponded closely with gill raker spacing of Atlantic salmon (Fig. 6*b*).

Discussion

The abundance and dry mass of organisms in the drift increased with current velocity and decreased with day of the year. As in our study, current velocity is usually the best predictor of the abundance of drift flowing through a cross-sectional area of stream (Elliott 1970; Everest and Chapman 1972; Wańkowski and Thorpe 1979; Smith and Li 1983; Grant and Noakes 1987). Seasonal declines in the abundance of drift,

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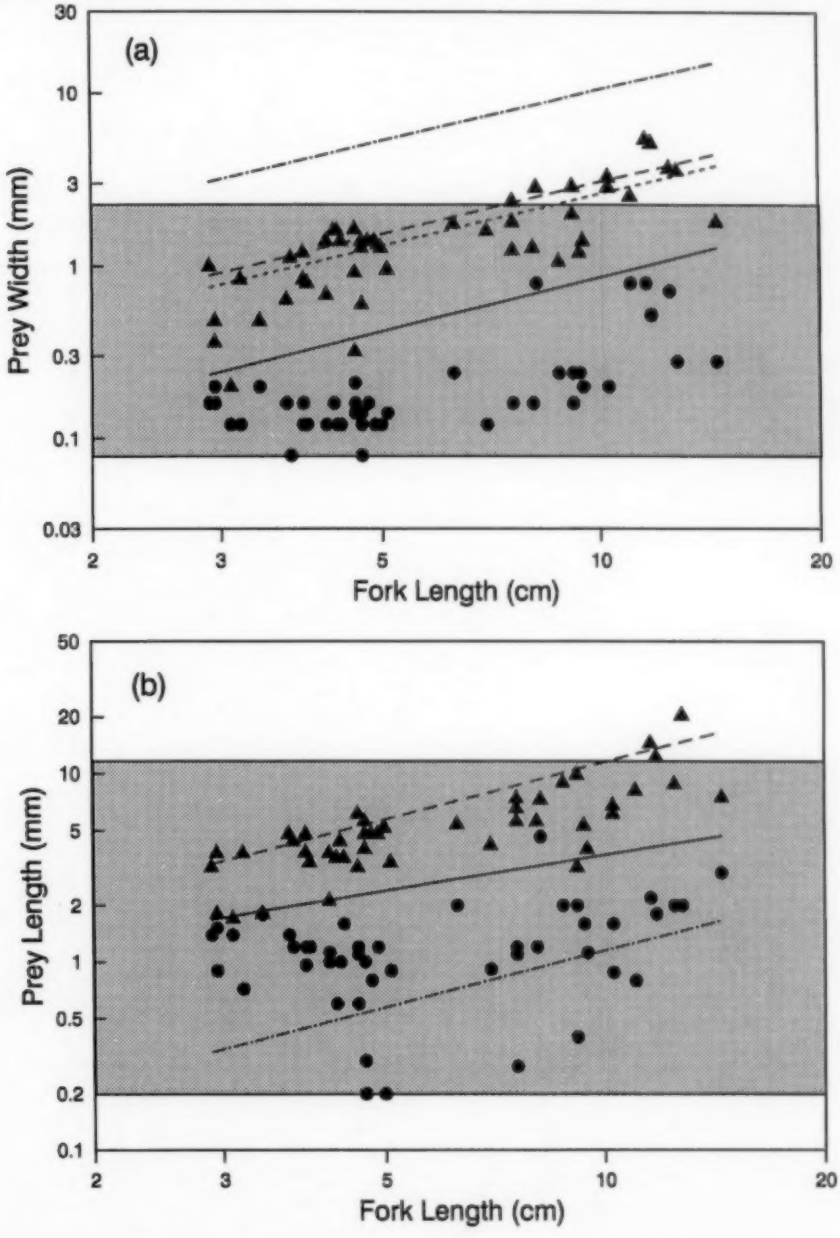
Fig. 6. (*a*) Minimum (circles), mean (solid line), and maximum (triangles) prey widths in relation to the fork length of 46 juvenile Atlantic salmon. The dashed–dotted line represents the maximum prey widths predicted by mouth dimensions (Wańkowski 1979) and the dashed and short-dashed lines represent the optimal prey widths predicted by Bannon and Ringler (1986) and Wańkowski (1979), respectively. The shaded horizontal band represents the range of invertebrate widths found in 46 drift samples, excluding the uppermost 5% of maximum and the lowermost 5% of minimum invertebrate widths. (*b*) Minimum (circles), mean (solid line), and maximum (triangles) prey lengths in relation to the fork length of 46 juvenile Atlantic salmon. The dashed line represents the optimal prey lengths predicted by Bannon and Ringler (1986) and the dashed–dotted line represents the minimum prey length predicted by gill raker spacing (Wańkowski 1979). The shaded horizontal band is as in (*a*) for invertebrate lengths.



primarily due to the emergence of adult insects, are also common in other studies (Brittain and Eikeland 1988; but see Grant and Noakes 1987). The size of organisms in the drift was unrelated to any environmental variable we measured; Grant and Noakes (1987) found a similar result.

Larger fish ate larger prey in our study, as in many earlier

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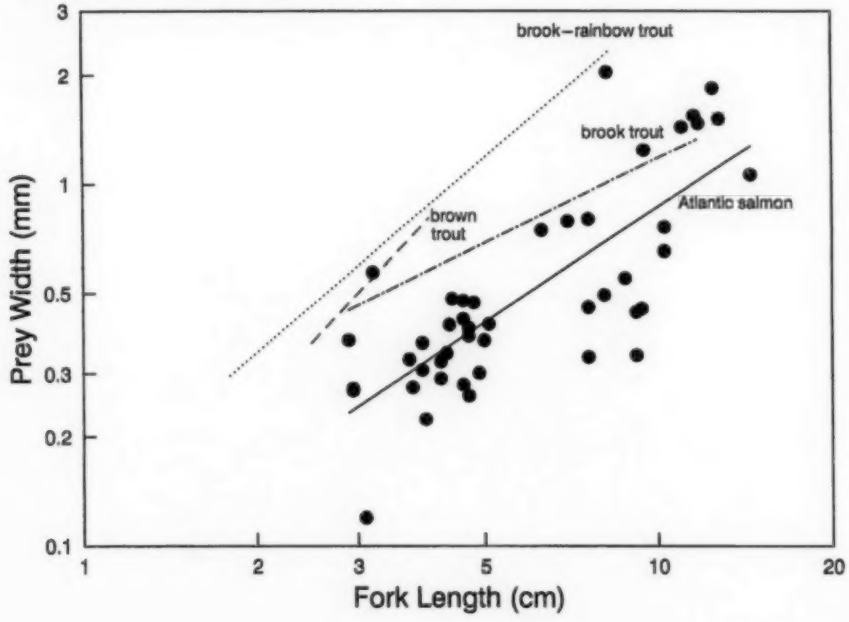
Table 4. Spearman's rank correlations (r_s) of the proportion of prey types in the drift versus the diet for stream-dwelling salmonids.

Species	r_s	P value	n^a	Reference
Oncorhynchus clarki	0.51	<0.2	10	Nakano et al. 1992
Oncorhynchus mykiss	0.81	<0.005	11	Angradi and Griffith 1990
Oncorhynchus mykiss	0.71	<0.1	7	Cada et al. 1987
Oncorhynchus mykiss	0.69	<0.2	7	Elliott 1973
Oncorhynchus kisutch	–0.10	0.78	10	Dunbrack 1992
Oncorhynchus tshawytscha	0.59	<0.05	13	Sagar and Glova 1987
Oncorhynchus tshawytscha	0.90	<0.002	10	Sagar and Glova 1988
Salmo trutta	0.79	<0.05	7	Cada et al. 1987
Salmo trutta	0.39	<0.5	7	Elliott 1973
Salmo trutta	0.49	<0.05	17	Glova et al. 1992
Salvelinus confluentus	0.39	<0.5	11	Nakano et al. 1992
Salvelinus fontinalis	0.46–0.68[b]	<0.05	25	Allan 1981
Salvelinus fontinalis	–0.25–0.69[c]	0.90–0.03	10	Grant and Noakes 1986
Salvelinus fontinalis	0.58	<0.05	18	McNicol et al. 1985
Salvelinus leucomaenis	0.86	<0.001	13	Furukawa-Tanaka 1985

[a] Number of taxonomic categories in the comparison.
[b] Five separate sampling dates and comparisons.
[c] Four separate sampling dates and comparisons.

Fig. 7. Least-squares regressions of prey width (mm) versus body length (cm) for brook trout and rainbow trout (dotted line, arithmetic equation: $Y = 0.30X - 0.25$, $r^2 = 0.95$; Rose 1986), brown trout (dashed line, arithmetic equation: $Y = 0.289X - 0.363$, $r^2 = 0.83$; Fahy 1980), brook trout (dashed–dotted line, $\log_{10} Y = 0.76 \log_{10} X - 0.72$, $r^2 = 0.45$; J.W.A. Grant, unpublished data), and Atlantic salmon (solid line and circles, $\log_{10} Y = 1.04 \log_{10} X - 1.11$, $r^2 = 0.61$; this study).



studies (Metz 1974; Newman and Waters 1984; Grant and Noakes 1986). Juvenile Atlantic salmon in Catamaran Brook primarily ate chironomid larvae, dipteran pupae and adults, and ephemeropteran and trichopteran larvae. These types of prey have also been reported in previous diet studies of juvenile Atlantic salmon (Allen 1941; Egglishaw 1967; Lillehammer 1973; Williams 1981; Thonney and Gibson 1989; Cunjak 1992).

Juvenile Atlantic salmon in Catamaran Brook fed opportunistically on all major types of invertebrates in the drift except for water mites. The similarity between the components of the drift and diet in our study has been noted in other studies of stream-dwelling salmonids (Table 4). Of the 22 correlation coefficients in Table 4, 19 were positive, and 12 were significantly so. In the three cases with negative correlations, the most abundant items in the drift, either water mites or ostracods,

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were rarely eaten (Dunbrack 1992; Grant and Noakes 1986). Other studies of Atlantic salmon (Egglishaw 1967) and coho salmon, *Oncorhynchus kisutch* (Dunbrack 1992) have also noted that water mites are not eaten. Water mites are often brightly coloured and may be unpalatable for fish (Kerfoot et al. 1980; Kerfoot 1982; but see Allan 1981).

Minimum, mean, and maximum prey size all increased with increasing body size of Atlantic salmon. However, the smallest and largest juveniles appeared to be the most selective foragers. Newly emerged young-of-the-year ate smaller prey than available in the drift, primarily small chironomid larvae, whereas large parr ate larger prey than available in the drift, primarily large dipteran pupae and adults.

Prey size selectivity in juvenile salmonids has often been linked to morphological constraints and to optimal prey size. Gill raker spacing was a good predictor of minimum prey length, supporting the hypothesis that morphological constraints set the lower limit of prey size ingested (Wańkowski 1979; Dunbrack and Dill 1983). Even if swallowed, age 2+ salmon may have been unable to retain small chironomids on their gill rakers. In contrast, the increase in mean prey size with body size probably had little to do with mouth size. Even newly emerged salmon should have been able to ingest about 95% of the available drift.

Feeding energetics may explain the increase in mean and maximum prey size with body size. Large prey contain more energy (Smock 1980) but take longer to handle (Bannon and Ringler 1986) than small prey. Salmon became increasingly size selective with body size, perhaps to maximize their net rate of energy gain. Despite this size-selective foraging, mean prey size in stomachs was much smaller than the optimum predicted by Wańkowski (1979) or Bannon and Ringler (1986). However, newly emerged parr appeared to prefer smaller prey than their predicted optimum. This discrepancy may result because the predicted optimal prey sizes are based on handling times for commercial pelleted food (Wańkowski 1979) and for crickets, *Acheta domestica* (Bannon and Ringler 1986). A resolution of this apparent discrepancy may require measuring the profitability (i.e., energy ingested per unit time) for each prey type and size in the drift.

Prey size versus body size relationships have been reported in three other studies of stream-dwelling salmonids. To compare these relationships, we replotted all four on a logarithmic scale; however, the original or arithmetic equations from Fahy (1980) and Rose (1986) are presented in the figure caption (Fig. 7). The allometric slopes for a combined brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) regression (Rose 1986) and for a brown trout regression (Fahy 1980) were greater than the slope for Atlantic salmon ($t = -4.95$, $P < 0.05$, and $t = -2.43$, $P < 0.05$; for brook–rainbow trout regression and brown trout regression, respectively). However, the allometric slopes for Atlantic salmon and brook trout (J.W.A. Grant, unpublished data) regressions did not differ (ANCOVA, $F_{1,119} = 3.35$, $P = 0.07$). It was also clear that the Atlantic salmon in our study ate smaller prey than similar-sized fish in the other studies. For example, the elevation of the brook trout regression was higher than the Atlantic salmon line (ANCOVA, $F_{1,119} = 10.38$, $P = 0.0016$). In addition, 45 of our 46 data points were below the brown trout regression (binomial test, $P < 0.0001$) and all 46 data points were below the brook–rainbow trout regression (binomial test, $P < 0.0001$).

The small size of the prey eaten by Atlantic salmon may be related to the fish's morphology or to the size distribution of invertebrates available in Catamaran Brook. Atlantic salmon parr have a smaller head, and perhaps a smaller gape, than brook, brown, or rainbow trout (Scott and Scott 1988). In addition, Atlantic salmon have more gill rakers than brown trout of a similar size (Hessen et al. 1988). Therefore, it is conceivable that Atlantic salmon may be limited by their morphology to eating smaller prey than the other species. However, three observations suggest that prey availability is a better explanation than morphological constraints for differences among the allometric relationships. First, based on mouth size (Wańkowski and Thorpe 1979), Atlantic salmon parr should have been able to eat the size of prey reported in Rose's (1986) study. Second, the marked differences between the two brook trout populations suggest environmental differences, such as the size of available prey. Third, the prey in Rose's (1986) study were not only larger but were taxonomically different from the prey in our study. For example, mayfly and stonefly nymphs composed a much larger proportion of the diet in his study than in our's. Given the opportunistic nature of salmonid foraging (Table 4), it seems likely that the size distribution of the drift in Rose's study was also larger than in our study.

In summary, juvenile Atlantic salmon were selective foragers, even though their diet resembled the taxonomic composition of the drift. Because of the small size of prey available, their diet was more likely constrained by gill raker spacing than by mouth size. The increasing size selectivity with increasing body size was likely due to salmon attempting to maximize their growth rate on prey of a relatively small size.

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Zebra mussel (*Dreissena polymorpha*) effects on sediment, other zoobenthos, and the diet and growth of adult yellow perch (*Perca flavescens*) in pond enclosures

Sarah A. Thayer, Robert C. Haas, R. Douglas Hunter, and Robert H. Kushler

Abstract: Zebra mussels (*Dreissena polymorpha*) in enclosures located in an experimental pond adjacent to Lake St. Clair, Michigan, increased sedimentation rate but had relatively minor effects on percent organic matter and percent nitrogen content of sediment. In contrast, sediment from Lake St. Clair adjacent to zebra mussels was significantly higher in carbon than that 0.5 m away. Zebra mussels increase the nutritional value of surficial sediment and provide greater structural heterogeneity, which is probably more important in causing change among zoobenthos. Zoobenthos and yellow perch (*Perca flavescens*) diet were dominated by dipteran larvae and leeches. Zoobenthos was significantly different between enclosures with and without zebra mussels. Treatments with zebra mussels had significantly more oligochaetes and tended to have more crustaceans (isopods and amphipods). In June, yellow perch without zebra mussels consumed significantly more zooplankton, and those with mussels had more crustaceans in their diet. Zooplankton density was greater in treatments without zebra mussels. Yellow perch with zebra mussels grew significantly more than those without mussels. Zebra mussels in the enclosures neither reproduced nor were eaten by yellow perch; hence. the observed growth differences were due to indirect effects involving zebra mussel induced changes in benthic structure and biota.

Résumé : Les moules zébrées (*Dreissena polymorpha*) dans des cages d'un bassin expérimental, voisin du lac St. Clair, au Michigan, ont augmenté de façon significative le taux de sédimentation, mais n'exerçaient que des effets relativement mineurs sur les pourcentages de matière organique et d'azote des sédiments. Par contre, des sédiments provenant du lac St. Clair, au voisinage des moules zébrées, renfermaient des teneurs en carbone significativement plus élevées que ceux situés 0,5 m plus loin. Les moules zébrées augmentent la valeur nutritionnelle des sédiments de surface et assurent une plus grande hétérogénéité structurelle, ce qui a probablement plus d'effet sur les changements au sein du zoobenthos. Le régime de ce dernier et de la perchaude (*Perca flavescens*) étaient dominés par les larves de diptères et les sangsues. Le zoobenthos était significativement différent entre les cages, en présence ou non de moules zébrées. Les traitements à l'aide des moules zébrées comportaient significativement plus d'oligochètes et avaient tendance à renfermer davantage de crustacés (isopodes et amphipodes). En juin, les perchaudes, sans présence de moules zébrées, consommaient significativement plus de zooplancton, et celles avec présence de moules avaient davantage de crustacés dans leur régime alimentaire. La densité du zooplancton était plus grande dans les traitements sans moules zébrées. Les perchaudes, avec présence de moules zébrées, avaient une croissance significativement supérieure à celles sans cette présence. Les moules zébrées dans les cages ne se reproduisaient pas et n'étaient pas consommées par les perchaudes; les différences observées dans la croissance étaient donc dues à des effets indirects associés aux changements induits par les moules zébrées dans la structure benthique et la biocénose.
[Traduit par la Rédaction]

Introduction

In addition to causing extensive economic damage, the exotic zebra mussel (*Dreissena polymorpha*) is also responsible for extensive ecological change on an ecosystem scale in areas of North American waters where it reaches high density (Nalepa and Fahnenstiel 1995). Documentation of these changes includes nutrients and water quality (Johengen et al. 1995; Fahnenstiel et al. 1995*a*); macrophytes (Skubinna et al. 1995); benthic algal community structure (Lowe and Pillsbury 1995); and the reduction of suspended particulate matter, including phytoplankton density (MacIsaac et al. 1991, 1992, Leach 1993, Holland 1993) and phytoplankton productivity (Fahnenstiel et al. 1995*b*). Although seston reduction may well have negative effects on higher consumers in food webs such as planktivorous fish, this has yet to be demonstrated. Another consequence of the zebra mussel invasion is that a number of benthic macroinvertebrate taxa respond to the presence of zebra mussels with higher abundance and biomass (Dusoge 1966; Dermott et al. 1993; Griffiths 1993; Slepnev et al. 1994; Stewart and Haynes 1994). Two mechanisms have been proposed to explain the positive response by these invertebrates.

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S.A. Thayer and R.D. Hunter.[1] Department of Biological Sciences, Oakland University, Rochester, MI 48309, U.S.A.
Robert C. Haas. Michigan Department of Natural Resources, Lake St. Clair Great Lakes Fisheries Research Station, 33135 South River Road, Mount Clemens, MI 48045, U.S.A.
Robert H. Kushler. Department of Mathematical Sciences. Oakland University, Rochester, Michigan 48309, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: hunter@oakland.edu

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One is that surficial sediments become nutrient-enriched because of large-scale biodeposition of seston converted to zebra mussel feces and pseudofeces, thereby providing infaunal invertebrates a greater quantity and possibly quality of food (Izvekova and Lvova-Katchanova 1972; Stanczykowska 1977; Walz 1978). Secondly, the development of dense aggregations of zebra mussels with a multitude of interstices increases the quantity and quality of habitat available to other invertebrates. More voluminous substrata provide a greater surface area for grazing by zoobenthos and more spatially heterogeneous substrata provide zoobenthos with shelter from predators. Spatial heterogeneity has been shown to be an important component in the coexistence of predators and prey (Huffaker 1958; Pimentel et al. 1963; Hardman and Turnbull 1974; and others). In this study, we predicted (H_1) that sedimentation rate would be greater in the presence of zebra mussels, as would the organic and nitrogen content of surficial sediment.

In the past, field studies to determine the effects of zebra mussels on other benthic invertebrates typically have been based on comparisons of current field data with data from years prior to the zebra mussel invasion, as well as data from different times of the year (Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994). Annual and seasonal effects inherent in such studies tend to be confounded with those that are due to the presence of zebra mussels. In contrast, this study was conducted using enclosures with and without zebra mussels in an experimental pond so that more direct simultaneous comparisons could be made between a zoobenthic community with and without the presence of zebra mussels. This study compares the zoobenthos in those enclosures, as well as enclosures without yellow perch (*Perca flavescens*). We predicted (H_2) that zebra mussel treatments (ZM) would have more zoobenthos (besides zebra mussels) than treatments without zebra mussels (NZM).

Zebra mussels may indirectly affect fish populations in two important ways. First, high densities of zebra mussels are thought to rapidly reduce phytoplankton and microzooplankton (MacIsaac et al. 1991, 1992; Leach 1993; Bunt et al. 1993; Holland 1993), a process that may enable them to outcompete larger zooplankton, resulting in lower food resources for planktivorous fish. However, this process is open to question as Wu and Culver (1991) found the opposite to occur: daphnids outcompeted zebra mussels. Second, increased productivity of benthic invertebrates may provide additional food resources for benthivorous fish, such as adult yellow perch, and elicit a positive growth response in fish. In this study, we tested this second effect by determining the stomach contents, length, and mass of adult yellow perch in pond enclosures, with and without zebra mussels. Adult yellow perch are primarily benthivores (Haas and Schaeffer 1992), particularly where the quality of the zoobenthos is high (Hayward and Margraf 1987; R.C. Haas, unpublished data). We hypothesized (H_3) that adult yellow perch would feed on a larger biomass of benthic invertebrates and (H_4) grow faster when zebra mussels were present than when zebra mussels were not present. Yellow perch are an abundant and widespread fish species native to North America. They are found in most inland lakes and in all of the Great Lakes, including many areas where zebra mussels have reached high density. Results of this pond study are applicable to yellow perch populations in larger water bodies, such as Lake St. Clair, where zebra mussels have reached an appreciable density.

Materials and methods

Experimental design

The following experiment took place May through October 1993 inside 18 enclosures that were fixed onto the bottom of a 0.15-ha, 1.0 m deep pond located near the northwest shoreline of Lake St. Clair (Fig. 1). Enclosures measured 1.8 × 1.8 × 1.5 m and had 0.63 cm mesh hardware cloth sides and lids. The mesh sides allowed water to infiltrate the enclosures as the pond was filled with water from Lake St. Clair on April 30, 1993. On this date, zebra mussel veligers were not detected in the water. Enclosure bottoms were made of fibreglass window screen that was covered with a mix of sediment and pea-stone gravel to a depth of 8 cm. Where applicable, zebra mussels clusters were placed onto this sediment surface. The bottom of every enclosure was divided into 24 quadrats, from which we randomly selected 8 to be the locations for sunken plastic trays that were filled with the same sediment–stone mix as the surrounding quadrats. The inside area of each tray measured 0.38 × 0.24 m, representing approximately 9% of 1.0 m^2. The trays served as a convenient way to quantitatively and consistently remove sediment from the enclosures for purposes of monitoring benthic invertebrate composition and evaluating zebra mussel mortality in enclosures containing zebra mussels. Trays were removed with specially constructed poles so that the other trays and the enclosure bottoms were not disturbed.

The following four treatments were used: (*i*) zebra mussels only; (*ii*) yellow perch only (NZM yellow perch); (*iii*) zebra mussels and yellow perch (ZM yellow perch); (*iv*) no zebra mussels or yellow perch (Fig. 2). Three replicates of treatments *i* and *iv* and six replicates of treatments *ii* and *iii* were used. Treatments were randomly assigned to enclosures within a blocked design (Fig. 2). Preliminary observations of water flow through enclosures and dissolved oxygen inside enclosures indicated that there were differences among enclosures that were surrounded by other enclosures (those near the northeast corner of the pond and those towards the center of the pond); therefore, three blocks were used, each of which consisted of enclosures with uniform conditions. Enclosures were blocked for similarity so we could control for potential location effects on yellow perch growth.

Clusters of zebra mussels, 5–33 mm (85% of the mussels were 13–23 mm), were collected from the western shoreline of Lake Erie on April 23, 1993, and held in a storage pond next to the experimental pond until April 30, when they were spread uniformly across the bottoms of appropriate enclosures at a density of approximately 8000 individuals·m^{-2}. The average zebra mussel density in nearby Lake St. Clair was reported to be 3200 individuals·m^{-2} in 1994 (Nalepa et al. 1996). Yellow perch ranging from 120 to 130 mm in total length (16–23 g wet mass) were collected by trawl, seine, and fyke nets from Lake St. Clair and placed into appropriate enclosures, six fish per enclosure. We selected 2-and 3-year-old yellow perch for this study, because they feed on a greater proportion of benthic invertebrates than younger or older yellow perch and, therefore, are most likely to be affected by changes in the composition of zoobenthos. A unique sequence of soft dorsal rays were removed from each yellow perch so that individual fish could be identified. Approximately 150 juvenile and adult yellow perch were stocked in the pond outside the enclosures to reduce the overall density of zooplankton and larger pond invertebrates (Hydracarina, larval and adult Coleoptera, Hemiptera (water boatmen and back-swimmers), and larval Pyralidae) to levels more typical of Lake St. Clair (a lower density of zooplankton; less Hydracarina; and virtually no Coleoptera, Hemiptera, or Pyralidae). High numbers of these invertebrates were found in the pond in 1992 and could have mitigated effects of zebra mussels in 1993.

Enclosure conditions were frequently monitored. Lids were kept closed when sampling was not taking place to exclude piscivorous birds. The sides of the enclosures were routinely cleaned with a wire brush to remove filamentous algae that could have reduced water flow through the enclosures. Pond temperature, dissolved oxygen, and

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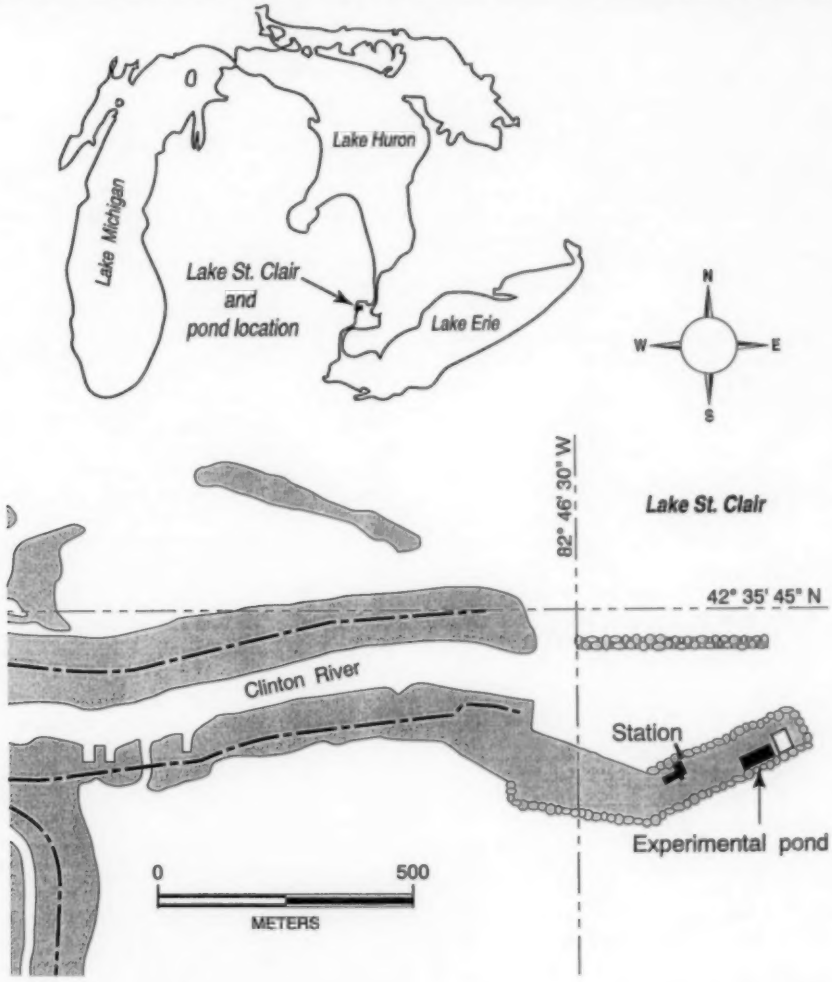
Fig. 1. Location of the experimental pond in southeastern Michigan at the outfall of the Clinton River adjacent to Lake St. Clair.

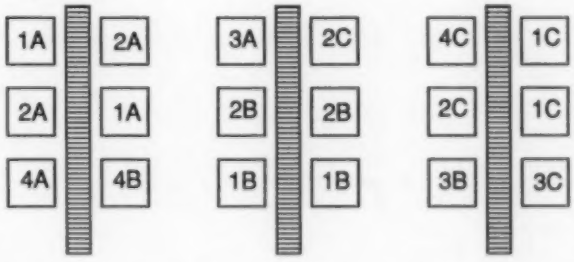


ammonia were measured two times each week near the pond bottom so that suboptimal conditions could be corrected by aerating the pond with an aspirator. Secchi disk transparency and pH were determined once each week and nitrogen (nitrate + nitrite) and phosphorus (total) once each month.

Zebra mussel growth and survival

Zebra mussel growth in the experimental pond was measured as the change in length and mass, May through October 1993 of 44 individual zebra mussels that were held within screened compartments of a singular Plexiglas™ unit (see Bitterman et al. 1994 for unit details). The unit was suspended in the pond outside one of the centrally located enclosures. We also counted live and dead (if any) zebra mussels in the unit in October to determine survival. The mussels used in the unit were collected from the mouth of the Clinton River. Their initial length and wet mass were 7.62 ± 0.015 mm (mean ± SE) and 0.06 ± 0.001 g. Because the Plexiglas™ unit was suspended in the water column, we also estimated zebra mussel mortality on the pond bottom inside the enclosures by visually inspecting the live and dead zebra mussels inside trays that were removed to sample zoobenthos in October. In preliminary studies in October 1992, we counted all the dead and live zebra mussels from one bottom tray of each of three

Fig. 2. Location of enclosures and arrangement of treatments around docks in the experimental pond: (1) zebra mussels only; (2) yellow perch only; (3) zebra mussels and yellow perch; (4) no zebra mussels or yellow perch; (A) block 1; (B) block 2; and (C) block 3.



enclosures without yellow perch and three enclosures with yellow perch to determine if yellow perch had an effect on zebra mussel mortality. We continuously inspected enclosures for new zebra mussel settlement and checked zooplankton samples for veligers because

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reproduction of zebra mussels may have seriously altered the experimental outcome.

Zooplankton

We determined the density and species composition of zooplankton in the experimental pond from zooplankton samples that were usually taken once each week with a 153-µm plankton net. Each sample consisted of a combination of three vertical plankton tows from three locations in the pond outside the enclosures or six vertical plankton tows in ZM and NZM enclosures. We did not expect to find differences in zooplankton density and species composition between ZM and NZM enclosures, because they were quite porous and different treatments were often within 1 m of each other.

Sediment analysis

We examined the effects of zebra mussels on sediments in the experimental enclosures by comparing altered sediment (ZM sediment) and sediment free of zebra mussel influence (control sediment) from three different sources. First, suspended particles were captured during settlement using two funnels to obtain a series of 12 pairs of samples from June 4 to October 4, 1993. Funnels measuring 20 cm in diameter were fixed in place next to each other at the end of the center dock 0.5 m below the water surface. Particles coming out of suspension over the funnels were collected in a glass vial at the lower end of the funnel. Over each funnel there was a Plexiglas™ unit consisting of 28 screened compartments (see Bitterman et al. 1994 for unit details). The Plexiglas™ unit on one of the funnels contained 28 zebra mussels (6–8 mm) that were collected from the mouth of the Clinton River. Hence, one funnel (control) collected normal particles while the other collected normal particles and particles generated by zebra mussels, including both feces and pseudofeces. Collected particles were initially frozen, then dried to a constant mass at 70 ± 5°C (mean ± SD) and stored frozen in glass. Subsamples of known mass were ashed in a muffle furnace at 490 ± 10°C for 1 h, and the mass of organic matter was obtained by subtraction. A second subsample was analyzed for total nitrogen using a Coleman model 29 nitrogen analyzer (Gustin 1960).

A second method of assessing ZM sediment utilized surficial sediments obtained from the upper 2 cm of trays from identical positions in each of the 18 experimental enclosures on October 21, 1993. These samples were frozen, dried, stored in glass, and analyzed for organic matter and nitrogen as above. Before analysis, all larger macroinvertebrates were removed by hand sorting.

A third method involved analysis of sediment collected on September 30, 1990, from two sites in Lake St. Clair located about 8 km north of the southern shore. (See central and eastern sites in Hunter and Bailey 1992.) Samples of sediment were collected from an approximate depth of 5 m by SCUBA divers who used 10-mL syringes to remove sediment from the top 1–2 cm. Sediment was collected at each site on, or immediately adjacent to, large mounds of zebra mussels and at distances of 0.5–1.0 m away from the nearest mound. These sediments were processed as those described for enclosures, except that organic carbon was measured using a wet oxidation method (Russell-Hunter et al. 1968).

Benthic invertebrate analyses

One plastic tray was removed every other month from the same randomly selected position in each enclosure of all four treatments. Sediment from each tray was reduced by passing it through a 250-µm sieve and separating out one quarter of the total amount using a Folsom splitter. To maintain homogeneous sediment and zebra mussel density over the bottom of the enclosures, replacement trays containing sediment, gravel, and zebra mussels (where applicable), were returned to enclosures. The subsample of sediment was preserved with 4% buffered formalin containing rose Bengal, a stain which facilitates the process of hand sorting macroinvertebrates from the samples. Oligochaeta, Ostracoda, and *Macrothrix* sp. were hand sorted from one sixteenth of the sample because of their high concentration.

All invertebrates in the subsamples were counted and identified as specifically as possible (usually to genus). At least 10 individuals of every genus in at least one enclosure in every treatment were measured using a stage micrometer (measurements accurate to the nearest 0.1 mm). Lengths of invertebrate taxa that were counted but not measured were estimated based on the mean size of the same taxa from the same tray or from the same treatment. This procedure accounted for 99% of the invertebrates contained in samples that were not directly measured. Invertebrates without assigned lengths were assigned a grand mean size for that invertebrate group. Benthic invertebrate measurements were taken from only one enclosure of each treatment in the fall because of time constraints.

Invertebrates were measured so they could be converted to a dry mass. Published length and mass regressions for each genus or a closely related genus were used (Nalepa and Quigley 1980; Culver et al. 1985; Meyer 1989; Theiling 1990). A dry mass regression for *Physella gyrina* was obtained from R.D. Hunter (unpublished data). Dry mass regressions for Coleoptera larvae and Hemiptera larvae and adults were formulated from measurements taken from this study, and because the two species of adult beetles found in the pond were both dytiscids and were within 1.0 mm in size of each other, one dry mass was determined and used for those individuals.

Invertebrate masses were combined into higher taxonomic groups for statistical analyses: microzoobenthos (Ostracoda and *Macrothrix* sp.), snails, caddisfly larvae, dipteran larvae (Chironomidae and Ceratopogonidae), leeches (primarily Erpobdellidae), flatworms, crustaceans (Amphipoda and Isopoda), oligochaetes, and "other" (water mites, larval Pyralidae, mayfly larvae, beetle larvae, adult Hemiptera, and larval Odonata). The "other" group consisted of invertebrates that are not exclusively benthic, but this group was included in statistical analyses nonetheless as these invertebrates were found in the benthic samples. Most of this group consisted of water mites.

Yellow perch diet and growth

We captured yellow perch inside enclosures monthly from June through October with a direct current electroshocking backpack unit and transported them to the laboratory 300 m away where standard length (to the nearest millimetre) and wet mass (to the nearest 0.1 g) were recorded and stomachs were immediately flushed by projecting a small stream of water from a squeeze bottle into their stomachs. Stomach contents were caught over a 153-µm sieve and preserved in 4% buffered formalin. Shocking always took place between 09:00 and 11:00 and yellow perch stomachs were pumped in random order. Yellow perch that died during the study were removed from enclosures and promptly replaced with new fish of similar size. Yellow perch were sacrificed and sexed in October.

At least 10 invertebrates of every genus in every stomach sample were measured except for a small portion of stomach samples collected in September and October. Invertebrates were measured and converted to dry mass as described for benthic invertebrates. Some of these invertebrates had been partially digested so that posterior sections of the invertebrate abdomens were absent. To obtain total body lengths of the more frequently encountered invertebrates, head capsules were measured and head-to-body regressions for *Gammarus* sp., *Caecidotea* sp., *Oecetis* sp., *Polycentropus* sp., and Chironomidae were formulated from measurements of whole individuals taken from this study so that heads widths (or depths) of partially digested individuals could be reliably converted to body lengths. Pearson correlation coefficients for all regressions were greater than 0.75.

Lengths of invertebrate taxa that were counted but not measured were estimated based on the mean size of the same taxon from the rest of the yellow perch stomachs from the same enclosure that month or from the yellow perch stomachs from the rest of the enclosures in that treatment for that month. This procedure accounted for 99% of the

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invertebrates contained in samples that were not directly measured. Invertebrates without assigned lengths were assigned a grand mean size for that invertebrate group.

For statistical analyses, invertebrates from yellow perch diet were combined into the same higher taxonomic groups as zoobenthos with the following differences: zooplankton was a group instead of microzoobenthos, and the "other" group also included flatworms and oligochaetes, despite their infrequent occurrence in yellow perch diet. As with the zoobenthic "other" group, water mites dominated. All references to invertebrates from benthos and yellow perch stomachs are in milligrams dry mass.

To determine if yellow perch selectively harvested prey relative to the abundance of prey that were available to them in the zoobenthos, we used Ivlev's electivity index (E; Diana 1995):

$$E = \frac{(R_i - P_i)}{(R_i + P_i)}$$

where R_i is the percentage of species i in the diet, and P_i is the percentage of species i in the environment. Ivlev's electivity index ranges between 0 and 1 for preference and between −1 and 0 for avoidance and was determined for the higher taxonomic groups described above and, additionally, for individual genera (or species) of invertebrates. Zooplankton was not included in this index because we expected the test yellow perch to feed primarily on zoobenthos.

Statistical analyses

A chi-square test was used to compare survival of zebra mussels in enclosures with and without yellow perch. A Wilcoxon signed-rank test was used to compare zooplankton density between NZM and ZM enclosures.

Paired t tests were used to compare particulate data from ZM and control enclosures as these data were obtained simultaneously at 12 different times. Analysis was done for sedimentation rate (mg dry sediment·day^{-1}), percent organic matter, and nitrogen content (μg nitrogen·mg dry mass^{-1}). Two sample t tests were used to analyze the tray sediment data, which consisted of simultaneous measurements from all 18 enclosures on October 21, 1993. Two-factor analysis of variance was used to analyze the Lake St. Clair sediment data, for which the factors were site location and distance from zebra mussel mound. There did not appear to be any significant interaction between site and distance from mound, so the additive analysis of variance (ANOVA) model was used. All tests on sediment were one-tailed tests, because we predicted sediment enhancement by zebra mussels.

Zoobenthos data were strongly skewed. Square-root transformation of the data made the data more normal and removed heteroscedasticity for some of the invertebrate groups. Those invertebrate groups that remained skewed tended to be groups that were highly significantly different from each other (e.g., oligochaetes; see Results). We determined ANOVA to be the best method of analyzing the zoobenthos data as nonparametric tests could not be used on a data set containing more than one factor (i.e., yellow perch, zebra mussels, and season). Multivariate analysis of variance was used to test the effects of treatment and season (spring, summer, and fall) on the zoobenthos. We were also interested in determining whether a difference between treatments exists for each individual variable. Because a single omnibus test (MANOVA) does not do this, each benthic invertebrate group was also tested individually using ANOVA. We used F tests based on "adjusted" sums of squares to account for unbalanced data. All tests were two tailed except for the test of treatment effect on total zoobenthos, which was a one-tailed test as we predicted total zoobenthic biomass to be greater in the presence of zebra mussels.

The Mann–Whitney rank-sum test was used to compare the dry mass of each benthic invertebrate group separately (two-tailed tests) and all invertebrate groups combined (one-tailed test), from NZM and ZM yellow perch stomachs, to determine if ZM yellow perch consumed a higher dry mass of invertebrates. Tests were performed for all months combined and separately for each month, because yellow perch diet can differ monthly (i.e., more zooplankton is typically available in the spring; some invertebrates can be preyed on heavily one month so that their numbers are greatly reduced the following month). We did not use a multivariate approach to analyze the stomach contents of NZM and ZM yellow perch for two reasons. First, the validity of traditional multivariate methods requires data that meet very stringent conditions, which our data did not. Second, we were interested in determining whether a difference between treatments exists for each individual variable, as was the case with the zoobenthos data.

Logistic regression (Hosmer and Lemeshow 1989) was used to compare survival of NZM and ZM yellow perch. Only yellow perch that were present at the beginning of the experiment and lived at least 1 month were used to determine growth. This was the most appropriate way to analyze fish growth, because yellow perch that were used as replacements usually lost mass after introduction into an enclosure for unexplained reasons, while the original yellow perch continued to maintain similar growth patterns as previous months (usually maintaining or adding mass). We did find, however, that the results of tests using all yellow perch were not significantly different from results of tests using the original yellow perch. In addition, growth data were examined for gender and enclosure location effects.

To assess the effect of zebra mussels on yellow perch growth, "growth curve" models were applied to the repeated length and mass measurements of the fish. Proper statistical analysis of such data must take account of the possible correlations between the repeated measurements on the same experimental unit (yellow perch). Using ordinary regression analysis would involve assuming that the repeated measurements are statistically independent, which is usually unrealistic. Traditional "split-plot repeated measures analysis" (Maceina et al. 1994) is based on a model called "compound symmetry," which assumes the same level of correlation between any two measurements on the same fish. This is usually unrealistic for growth curve data, for which it is reasonable to assume that the correlation is stronger for measurements that are close together in time and weaker if the time between the two measurements is longer. The most general approach, multivariate regression, allows an arbitrary correlation structure among the repeated measurements but can be inefficient and is quite sensitive to the presence of outliers in the data.

New software now provides greater flexibility for modeling growth curve data. "Random coefficient models" assume the growth-curve parameters vary randomly among individual fish within a treatment group, with treatment effects, if any, causing systematic changes in the parameters between groups. The variation in the growth curve parameters among individuals within a group (and in this study, additional variation due to a random "enclosure effect") is taken into account when testing for differences between groups, and the model produces a realistic correlation pattern for the repeated observations. The software also accommodates unbalanced data sets with missing observations, which is important as some of the yellow perch were not retrieved in all months and several died before the experiment ended.

Procedure Mixed in release 6.11 of SAS (Littell et al. 1996) was used to perform these analyses. This program uses a generalized least-squares algorithm to estimate the growth curve parameters for each treatment group, the variances and covariances of the random coefficients, and the measurement errors. Tests of the significance of treatment effects are performed using statistics that have an approximate F distribution if the sample size is sufficiently large and the assumed model is correct. The random noise is assumed to be normally distributed, and the usual "equal variances" assumption becomes "equal covariance structures." We examined residuals to assess the normality assumption, and tested equality of the covariance structure between ZM and NZM groups. One-tailed tests were used to compare yellow perch growth between ZM and NZM treatments, because we predicted ZM yellow perch would grow faster. Two-tailed tests were used to compare initial sizes and mortality of ZM and NZM yellow

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Table 1. Zooplankton densities (no.·L^{-1}) outside and inside experimental pond enclosures.

Date	Outside enclosures	Inside enclosures	
		NZM	ZM
Apr. 1993		2.3	0.5
May 1993		95.0	89.0
		8.5	13.0
		25.0	17.0
June 1993		15.0	13.0
		4.2	4.1
July 1993	113.0	59.0	34.0
Aug. 1993		174.0	112.0
Sept. 1993	89.0		
Oct. 1993	10.0		

Note: Zooplankton density in enclosures with no zebra mussels and no fish (NZM) is significantly higher than in enclosures with zebra mussels and no fish (ZM) (Wilcoxon signed-rank; $Z = -1.96$; $P = 0.049$).

perch and growth between ZM and NZM yellow perch when enclosure location and gender were considered.

Alternative assumptions about the correlation structure (i.e., the various approaches discussed above) can be compared and tested using likelihood ratio statistics, which have an approximate chi-squared distribution in large samples. (These statistics are also used to test the "equal covariance structures" assumption.) We considered several alternatives, ranging from compound symmetry to the fully general multivariate approach. In almost all cases the random coefficient model described above was the best choice, based on the likelihood ratio test statistics. In some cases an additional term accounting for serial correlation of the random noise in the data was needed.

We used a simple straight-line growth model for all analyses on yellow perch growth; hence, there are two growth curve parameters: the initial mass or length (which should not differ between treatment groups since the fish were randomly assigned) and the slope or growth rate. While it is likely that the actual growth pattern is nonlinear, the available data are not sufficient for fitting more complicated models. The simple linear trend model appears to be a reasonable approximation, and our substantive conclusions concerning treatment effects would almost certainly be the same if a different growth curve model were used.

Whenever more than one statistical test is performed, the probability of at least one "false positive" result (i.e., obtaining a small P value even though the effect being tested is negligible or nonexistent) is higher (much higher, if many tests are performed) than the nominal level of significance. One solution to this problem is to adjust the threshold for declaring statistical significance using the Bonferroni inequality. This requires dividing the threshold value by the number of tests performed. We believe choosing a specific threshold is arbitrary; however, the Bonferroni adjustment can be used as a guideline to assess the strength of our results when a number of statistical tests have been performed.

Results

Pond water quality

Pond temperature averaged 19 ± 4.3°C (mean ± SE), May 1 – October 15 ($N = 20$). Dissolved oxygen concentration near the bottom was 8–14 mg·L^{-1}, except for a low value of 4.6 mg·L^{-1} in mid-June and another low of 3.3 mg·L^{-1} in mid-July. These low oxygen levels lasted several days, so we initiated artificial aeration with an aspirator. Bottom ammonia levels were 0.08–0.84 mg·L^{-1}, with the highest levels occurring prior to June 15 (initial mortality of newly introduced zebra mussels).

Monthly chemical analyses consistently showed trace amounts (<0.01 mg·L^{-1}) of nitrate + nitrite, approximately 0.05 mg·L^{-1} total phosphorus, and pH values ranging between 8 and 10.5. In comparison, Lake St. Clair averaged 0.32 mg·L^{-1} nitrate + nitrite and 0.02 mg·L^{-1} total phosphorus from the end of April through the beginning of July 1990–1994 (R.C. Haas, unpublished data). Secchi disk readings averaged 0.75 ± 0.160 m in the pond.

Zebra mussel growth and survival

All 44 of the zebra mussels inside the suspended Plexiglas™ unit survived from May 12 to October 4, 1993. Survival of zebra mussels on enclosure bottoms, as determined from visual inspection of zebra mussels from bottom trays removed from each and every enclosure that contained zebra mussels ($N = 9$), was estimated in October to be 80–85%. We compared survival of zebra mussel in treatments with and without yellow perch by counting live and dead zebra mussels in one tray from each enclosure with zebra mussels and yellow perch ($N = 3$) and each enclosure with zebra mussels and no fish ($N = 3$). Survival of mussels was higher in the treatment without yellow perch ($\chi^2 = 10.10$, df = 1, $P = 0.001$); however, survival of mussels was also different among enclosures ($\chi^2 = 23.93$, df = 1, $P < 0.001$). When we included variation among enclosures in the treatment test by adjusting for overdispersion, zebra mussel survival was not different between treatments with and without yellow perch ($\chi^2 = 1.69$, df = 1, $P = 0.19$).

The lengths of zebra mussels inside the suspended Plexiglas™ unit increased from 7.62 ± 0.102 to 15.86 ± 0.196 mm. The 95% confidence interval of the difference in length is 7.833–8.642 mm. Wet mass increased from 0.06 ± 0.003 to 0.54 ± 0.019 g. The 95% confidence interval of the difference in mass is 0.449–0.525 g.

There was no evidence that zebra mussels reproduced in the pond. Settlement of small zebra mussels was not detected nor were veligers found in zooplankton samples.

Zooplankton

Zooplankton density ranged from 0.5 individuals·L^{-1} in April to 174.0 individuals·L^{-1} in August (Table 1). Zooplankton densities were measured separately by treatment, with and without zebra mussels, nine times during the study (Table 1). Zooplankton density was higher in the NZM treatment than in the ZM treatment ($Z = -1.96$, $P = 0.049$). Species composition was similar between treatments at almost all times; however, the mean mass of certain dominant species on some dates was sometimes lower for zebra mussel treatments.

Zooplankton density was often high, as it was in preliminary studies when yellow perch were not stocked outside the enclosures; however, species composition May through July was dominated by Copepoda and was heavily dominated in August through September by *Bosmina longirostris*. Daphnids were rare and never present in large numbers. Preliminary zooplankton tows in 1992 indicated daphnids were nearly always present and *B. longirostris* was less prevalent. Yellow perch placed outside enclosures in 1993 probably reduced daphnid numbers. Preliminary measures of zooplankton in the pond in 1992 indicated a high density of the dominant daphnid, *Ceriodaphnia* sp., (17.0 ± 3.88 individuals·L^{-1}) relative to that

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Table 2. Qualitative and quantitative measures (mean ± SE) of particulates and tray sediments without zebra mussels (NZM) and with zebra mussels (ZM).

Treatment	Sedimentation rate (mg·day^{-1} afdw)	Percent organic matter	Nitrogen (μg N·mg^{-1} dw)	Carbon (μg C·mg^{-1} dw)
Particulates				
NZM	86±130	16.80±0.620	9.72±0.528	
ZM	169±370	14.70±0.720	8.39±0.683	
Tray sediments				
NZM		5.62±0.057	3.05±0.318	
ZM		8.60±1.497	3.80±0.878	
Lake sediments				
Adjacent			3.04±0.386	21.40±2.093
Far			1.86±0.024	15.30±0.835

Note: Lake St. Clair data are for sediment collected adjacent to and far from (at least 0.5 m) a zebra mussel mound. afdw, ash-free dry weight; dw, dry weight.

found in the pond in 1993 (3.3 ± 1.69 individuals·L^{-1}). A lower density of daphnids is more representative of conditions in lakes such as Lake St. Clair.

Sediment analyses

The 28 caged zebra mussels over the 20 cm diameter funnel are approximately equivalent to a density of 900 individuals·m^{-2}, which is a relatively low density compared with either the experimental pond or Lake St. Clair. Zebra mussels growing in cages averaged 7.62 ± 0.678 mm starting length and by October had grown to an average length of 15.86 ± 1.301 mm. There was a tendency for zebra mussels over funnel particulate traps to increase the rate of sedimentation ($t_{(11)} = 2.46$, $P = 0.01$) by approximately 96% (Table 2). Trapped particles under the zebra mussels tended to be slightly lower in organic matter ($t_{(11)} = 2.01$, $P = 0.04$); however, nitrogen content did not differ between ZM sediment and the control ($t_{(11)} = 1.39$, $P = 0.10$).

Sediment samples from ZM trays tended to be higher in organic matter and nitrogen content than samples from NZM trays ($t_{(16)} = 1.86$, $P = 0.04$ and $t_{(16)} = 1.74$, $P = 0.05$, respectively; Table 2). Lake sediment samples adjacent to mounds of zebra mussels tended to have higher nitrogen and had significantly higher organic carbon content compared with sediments collected from at least 0.5 m away from such mounds ($F_{(1,5)} = 8.26$, $P = 0.02$ and $F_{(1,5)} = 26.82$, $P = 0.002$, respectively; Table 2). Samples were taken at two sites 9.5 km apart. Carbon was different between the two sites ($F_{(1,5)} = 16.53$, $P = 0.005$) but nitrogen was not ($F_{(1,5)} = 0.36$, $P = 0.29$).

Benthic invertebrate analyses

Two benthic samples from July were lost due to inadequate fixation. Analyses of invertebrate biomass by treatment showed that dipteran larvae and leeches were strongly dominant over other macroinvertebrate groups (Fig. 3A). There was a slight tendency for NZM treatments to have higher dry mass of dipteran larvae, and for leech dry mass to be lower in treatments with yellow perch. Treatment effects on other zoobenthic groups are more apparent when these dominant groups are omitted (Fig. 3B).

Results of MANOVA indicated differences in zoobenthos by month ($F_{(28,32)} = 14.21$, $P < 0.001$) and differences in zoobenthos between ZM and NZM treatments ($F_{(14,15)} = 24.04$, $P < 0.001$). No differences in zoobenthos between treatments with and without yellow perch were found ($F_{(14,15)} = 1.16$, $P = 0.39$).

Individual ANOVA on each benthic invertebrate group (Table 3) indicated there were more oligochaetes in ZM treatments ($F_{(1,28)} = 296.32$, $P < 0.001$). Crustacea tended to be higher in ZM treatments ($F_{(1,28)} = 5.39$, $P = 0.03$) and "other" invertebrates tended to be higher in NZM treatments ($F_{(1,28)} = 5.39$, $P = 0.03$). The only difference between treatments with and without yellow perch was that leeches tended to be higher in treatments with yellow perch ($F_{(1,28)} = 4.86$, $P = 0.04$). The dry mass of all groups combined (total) did not differ between NZM and ZM treatments ($F_{(1,28)} = 1.42$, $P = 0.12$), or between treatments with and without yellow perch ($F_{(1,28)} = 3.38$, $P = 0.08$).

Yellow perch diet

We found pumping yellow perch stomachs to be an effective means of stomach content removal. Periodic testing of this procedure by examining stomachs of yellow perch that died during the study period or that were sacrificed at the end of the year indicated that very little stomach content remained following pumping.

Yellow perch diet is summarized in Fig. 4. Stomach contents were fairly similar in dry mass between treatments except in June, when ZM yellow perch had over 50% more in their stomachs than NZM yellow perch. Zooplankton was a relatively minor diet component except in June when it was higher in NZM yellow perch stomachs. Overall, the diets were heavily dominated by dipteran larvae, except in October when leeches were dominant.

The results of rank-sum tests on yellow perch diet data indicated no difference in total dry mass of all invertebrate groups combined between NZM and ZM yellow perch ($Z = 0.53$, $P = 0.30$). When invertebrate groups were analyzed individually, NZM yellow perch tended to have more zooplankton per stomach ($Z = 2.18$, $P = 0.03$), most significantly in June ($Z = 2.84$, $P = 0.005$). Yellow perch with zebra mussels tended to have more Crustacea per stomach ($Z = 1.62$, $P = 0.07$) in June, and NZM yellow perch tended to have more snails per stomach ($Z = 1.64$, $P = 0.05$) in August. Mean and maximum dry mass of each invertebrate group per yellow perch stomach were quite similar between treatments, except for crustaceans and "other" invertebrates, which were two to three times higher in ZM yellow perch stomachs. Epiphytic invertebrates

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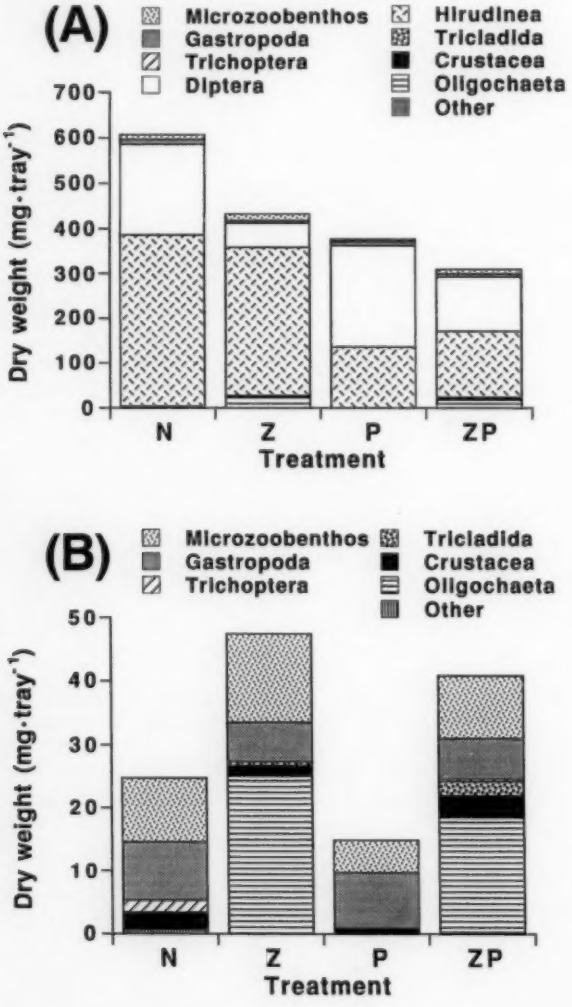
Fig. 3. Zoobenthos biomass per tray by treatment and taxonomic group for all groups (A) and all groups except Diptera and Hirudinea (B). N, Enclosures without zebra mussels or yellow perch; Z, enclosures with zebra mussels but no yellow perch; P, enclosures with yellow perch but no zebra mussels; ZP, enclosures with both zebra mussels and yellow perch.



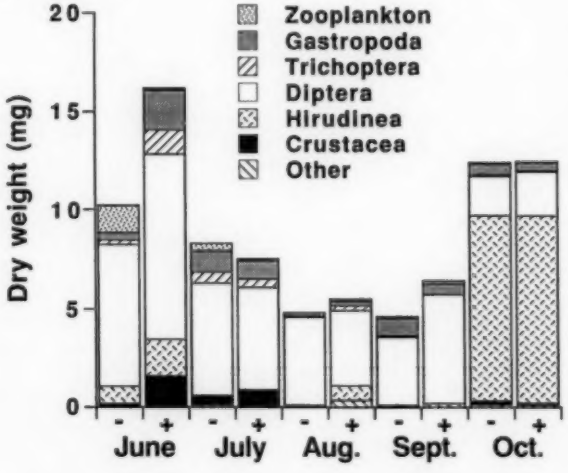
were rarely found in the stomachs of the test yellow perch; thus, prey availability was similar to that found in lakes such as Lake St. Clair. No zebra mussels were found in the stomachs of any of the test yellow perch.

Ivlev's electivity index increased and approached (or reached) 1.000, June through October, for *Gammarus* sp., *Physella gyrina*, *Procladius* sp., and *Tanytarsus* sp. for both NZM and ZM yellow perch. Yellow perch with zebra mussels had a higher electivity index for *Procladius* sp. in spring, summer, and fall (0.580, 0.484, 1.000, respectively) than NZM yellow perch (–0.510, –0.442, 0.680, respectively), and water mites (ZM yellow perch = 1.000, 0.991, 0.866, respectively; NZM yellow perch = 0.307, 0.491, 0.095, respectively). Yellow perch with zebra mussels had a higher electivity index for *Macrothrix laticornis* in summer and fall (0.543 and 0.973) than NZM yellow perch (–0.219 and –0.067). Yellow perch from both treatments had negative electivity indices for *M. laticornis* in spring. Yellow perch with zebra mussels had a higher electivity index for *Caecidotea* sp. in spring and fall (0.782 and 0.894) than NZM yellow perch (–0.345 and 0.000), although it was higher for NZM yellow perch (1.000) than ZM yellow perch (–0.172) in summer. Yellow perch with zebra mussels had higher electivity indices for *Ablabesmyia* sp. in spring and summer (1.000 for both) than NZM yellow perch (–0.346 and 0.138), but both equaled 1.000 in fall. Ivlev's electivity index was never greater for any invertebrate in a NZM yellow perch for more than one season and was usually smaller or equivalent to a ZM yellow perch.

Table 3. Mean (±SE) dry mass (mg) for zoobenthos in trays for different treatments (without (NZM) and with (ZM) zebra mussels) and results of analysis of variance.

Taxon	NZM	ZM	$F_{(1,28)}$	P
Microzoobenthos	6.89±3.040	11.45±3.903	0.49	0.49
Snails	9.01±3.543	6.34±2.024	0.02	0.90
Trichoptera	0.82±0.533	0.25±0.194	1.83	0.19
Diptera	216.69±35.235	94.28±38.532	3.28	0.08
Leeches	222.42±50.538	220.67±55.619	0.52	0.48
Flatworms	0.08±0.051	1.59±1.115	1.11	0.30
Crustacea	0.87±0.531	2.72±1.193	5.39	0.03
Oligochaeta	0.21±0.096	20.92±2.208	296.32	<0.001
Other	0.49±0.225	0.24±0.074	7.43	0.01
Total	457.47±64.906	358.46±63.519	1.42	0.12

Fig. 4. Mean biomass of yellow perch stomach contents by taxonomic group. Bars with minus signs show yellow perch without zebra mussels and with plus signs show yellow perch with zebra mussels.



Yellow perch survival and growth

Yellow perch survival in the enclosures was moderate. Approximately 50% of the original yellow perch died. Cause of death was either undetermined or thought to be from excessive electroshocking. The total mortality rate of yellow perch in

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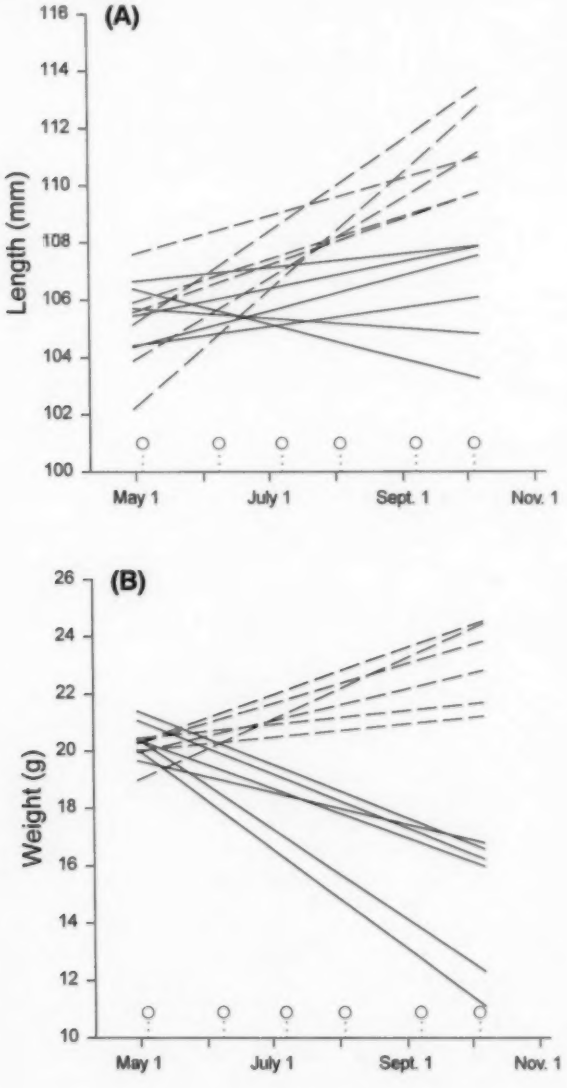
Fig. 5. Estimated growth curves for change in standard length (A) and wet weight (B) for all original yellow perch by enclosure. Solid lines represent enclosures without zebra mussels and broken lines represent enclosures with zebra mussels. Open circles show actual sample dates.



each enclosure did not differ by treatment, with and without zebra mussels, ($\chi^2 = 0.01$, df = 1, $P = 0.91$) nor by enclosure ($\chi^2 = 13.22$, df = 10, $P = 0.21$).

We were able to capture most of the test yellow perch each month to measure them and obtain their stomach contents. Those that were not captured either could not be located in the enclosure due to poor water visibility or had died and not floated to the surface. Different numbers of yellow perch were retrieved from each enclosure, each month, but the total number of yellow perch captured was distributed evenly between treatments.

The lengths and mass of yellow perch at the start of the experiment (April) were not different between treatments ($F_{(1,62)} = 0.20$, $P = 0.66$ and $F_{(1,62)} = 0.36$, $P = 0.55$, respectively). Changes in length (Fig. 5A) and mass (Fig. 5B) of ZM yellow perch were greater than that of NZM yellow perch (Table 4; $F_{(1,10)} = 19.10$, $P < 0.001$ for length; $F_{(1,62)} = 55.02$, $P < 0.001$ for mass).

Table 4. Mean initial size (standard length and wet mass) and mean daily growth (change in standard length and wet mass per day) of yellow perch as determined from the weighted average slope of all the yellow perch growth curves within each treatment with (ZM) and without (NZM) zebra mussels.

	Initial size		Daily growth	
Treatment	SL (mm)	Mass (g)	ΔSL per day (mm)	Δmass per day (g)
Both sexes				
NZM	105.30	20.20	0.004	–0.036
ZM	105.01	19.98	0.038	0.020
Females				
NZM	106.36	21.69	0.008	–0.104
ZM	103.54	19.41	0.061	0.042
Males				
NZM	105.35	20.16	0.002	–0.035
ZM	105.52	19.97	0.026	0.012

Growth was analyzed separately with sex as an additional factor, and in a separate analysis, by block location. Gender did not change the results of growth analysis as both female and male ZM yellow perch gained mass and both female and male NZM yellow perch lost mass. The changes in length and mass of ZM yellow perch were still greater than that of NZM yellow perch (treatment $F_{(1,10)} = 21.26$, $P = 0.001$ for length; $F_{(1,10)} = 41.42$, $P < 0.001$ for mass). The changes in length and mass of females from both treatments were greater than that of males from both treatments (gender $F_{(1,10)} = 10.19$, $P = 0.002$ for length; $F_{(1,10)} = 5.82$, $P = 0.02$ for mass). The treatment effect was greater for females (gender × treatment $F_{(1,92)} = 5.23$, $P = 0.02$). In addition, enclosure location did not change the results of the growth analysis (treatment $F_{(1,6)} = 30.80$, $P = 0.001$ for length; $F_{(1,6)} = 68.37$, $P < 0.001$ for mass). Enclosures were blocked for similarity, and we found that the change in length of yellow perch was different among blocks ($F_{(2,107)} = 6.35$, $P = 0.003$) but that their change in mass was not ($F_{(2,107)} = 2.31$, $P = 0.10$). Treatment effects did not differ among the three blocks (block × treatment $F_{(2,107)} = 0.99$, $P = 0.38$ for length; $F_{(2,107)} = 0.10$, $P = 0.90$ for mass).

Discussion

In our analyses of the food web presented in Fig. 6 we placed emphasis on the interaction between zebra mussels and yellow perch to underscore the dominant role played by the former in energy transfer. We did not demonstrate all of the interactions in this diagram and did not find any zebra mussels in the stomachs of the test perch, although zebra mussels have been found in stomachs of a small percentage of yellow perch in Lake St. Clair (personal observations)

In areas where zebra mussels are moderately dense (>2000 individuals·m^{-2}), they facilitate the energy transfer from the planktonic to the benthic sector of the lake ecosystem. Surficial sediments become thicker and possibly enriched,

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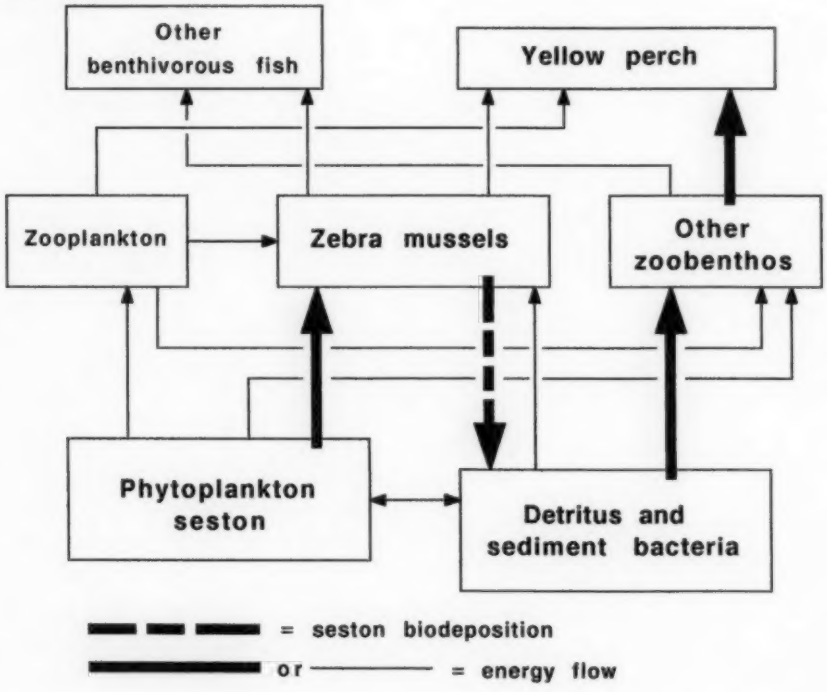
Fig. 6. Food web diagram based on data from this study and field observations showing the major taxonomic groups that interact with zebra mussels. Seston biodeposition is shown as a broken line to distinguish it from all other trophic interactions, which are solid lines. Arrows in boldface indicate the major energy pathway.



because of an enhanced bacterial community in the sediment. The quantity and quality of spatial heterogeneity is enhanced by the thicker and looser nature of the sediment as well as the added structure of zebra mussel clusters. A recent study (T.W. Stewart, Department of Biology, Bowling Green State University, Bowling Green, OH 43403, unpublished data) indicates that structural (interstices) rather than nutritional enhancement is the primary mechanism by which zebra mussels promote change in macroinvertebrate abundance, biomass, and community structure. Griffiths (1993) suggested zebra mussel feces and pseudofeces were responsible for increased abundance of several species of tubificids in southeastern Lake St. Clair following invasion by zebra mussels. Similarly, Izvekova and Lvova-Katchanova (1972) claimed that greater productivity in chironomids was due to an increase in organic matter produced by zebra mussels. The amount of organic material in sediment was positively correlated with abundance and mean size of several groups of benthic invertebrates by Cole and Weigmann (1983). On the other hand, Griffiths attributed higher numbers of *Gammarus*, flatworms, snails, *Polycentropus*, and *Polypedilum* to a habitat shift towards greater spatial heterogeneity and Dermott et al. (1993) suggested that *Gammarus*, *Caenis*, *Erpobdella punctata* and *Helobdella stagnalis* benefited from the habitat structure of zebra mussels. The significance of sediment organic content and substrate structure is likely to be species specific. Slepnev et al. (1994) placed real zebra mussels and rubber models of zebra mussels inside separate perforated cuvettes in cooling ponds at the Krivoy Rog and Chernobyl power plants and found more gammarids, ostracods, and oligochaetes in cuvettes with zebra mussels, but equal amounts of snails in both treatments, suggesting substrate was a greater influence on habitat preference by snails but not necessarily to other invertebrate groups. In this study, we found ZM yellow perch to have a greater frequency of high electivity indices for almost every zoobenthic group than NZM yellow perch, suggesting zoobenthos was more accessible to ZM yellow perch, possibly because of a more heterogeneous substratum produced by aggregations of zebra mussels. Water mites, *Macrothrix laticornis*, *Caecidotea* sp., *Procladius* sp. and *Ablabesmyia* sp. all had greater electivity indices for ZM yellow perch. In this case, structural enhancement by zebra mussels provided these invertebrates with more grazing area rather than protection from predators.

Slepnev et al. (1994) found six times more sedimentation in cuvettes with real mussels but a similar amount of organic component in both treatments. The pond data agree with this assessment. Sedimentation rate tended to be greater in sediment traps containing zebra mussels; however, we did not find significant differences in either percent organic matter or in nitrogen content. Organic matter and carbon had a tendency to be higher in ZM tray sediments, but this tendency was weak (i.e., $P = 0.04$ and 0.05, respectively). Lake sediment data provide a somewhat different impression: at high zebra mussels densities, enhancement of carbon and nitrogen may occur. Samples of sediment adjacent to mounds of zebra mussels in Lake St. Clair are comparable with high-density conditions, and these indicate enrichment in carbon. More distant samples were minimally influenced by zebra mussel feces and pseudofeces

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tended to be lower in nitrogen and were significantly lower in carbon ($P = 0.004$). Hence under field conditions it appears that high densities of zebra mussels (ca. ≥10 000 individuals·m^{-2}) may indeed create sediment conditions leading to carbon (and possibly nitrogen) enrichment. Nitrogen enrichment of sediments associated with zebra mussels compared with sediments without zebra mussels was also observed by Marvin and Howell (1996) at nearshore sites in Lake Erie and Lake Ontario. It is important to note that, even when the percentage of organic matter is essentially equal between NZM and ZM treatments, ZM treatments still have more total organic matter because sedimentation rate is higher. The accessibility of this organic matter to invertebrates is another important consideration. If invertebrates are capable of utilizing vertically distributed organic matter, they may benefit from the additional sediment. Zebra mussels may also make the organic matter in sediment more available to zoobenthos by production of mucous-bound pellets that provide access via interstices. Additionally, Slepnev et al. (1994) found that unbound precipitated matter on rubber models of zebra mussels washed off after several days, while bound matter on zebra mussels remained intact. Our data suggest that zebra mussels can change the benthic macroinvertebrate community through quantitative and qualitative nutritional enhancement of surficial sediment and increased structural heterogeneity. The relative importance of these two enhancements is likely to be species specific. In general, a modest level of nutritional improvement would suggest a majority of taxa respond to structural enhancement.

The zebra mussels in this study altered the zoobenthos, as indicated by a difference among the groups of zoobenthos between ZM and NZM treatments ($P < 0.001$), although the total biomass of invertebrates was not different between ZM and NZM treatments ($P = 0.24$). The results were not compromised by settlement of zebra mussels in NZM enclosures as shallow ponds are subject to diurnal temperature fluctuation that is known to cause physiological stress to zebra mussels sufficient to prevent gonad maturation and spawning (Borcherding 1992; Smit et al. 1992, 1993).

We found more oligochaetes in ZM treatments ($P < 0.001$) than in NZM treatments. Other studies have observed that zebra mussels have a strong and consistently positive effect on oligochaetes (Dermott et al. 1993; Griffiths 1993; Slepnev et al. 1994; Stewart and Haynes 1994). Apart from oligochaetes, "other" invertebrates, which primarily consisted of water mites, tended to be higher in the NZM treatments; however, in the absence of yellow perch, this tendency may have not occurred. Yellow perch with zebra mussels had a higher electivity index for water mites than NZM yellow perch. We did not find more "other" invertebrates in ZM yellow perch diet than in NZM yellow perch diet ($P = 0.60$); however, water mites tended to be more abundant in ZM yellow perch stomachs than in NZM yellow perch stomachs in August (9.35 ± 6.201 and 2.90 ± 1.512, respectively), and in September (7.41 ± 6.143 and 4.67 ± 3.528, respectively). Water mites may have been more susceptible to predation in ZM treatments.

There was tendency for crustacean dry mass to be higher in ZM treatments. We believe this to be a noteworthy result, because there was also a tendency in June for ZM yellow perch to have more crustaceans in their stomachs. In addition, other studies have found more crustaceans in the presence of zebra mussels (Dusoge 1966; Dermott et al. 1993: Griffiths 1993; Slepnev et al. 1994; Stewart and Haynes 1994). Amphipods were a major component of the diet of adult yellow perch in Lake Erie (Danehy et al. 1991), Lake St. Clair (Synnestvedt 1997), and Saginaw Bay (Haas and Schaeffer 1992). Yellow perch in our study preferentially preyed upon *Gammarus* sp., as well as *Physella gyrina*, *Procladius* sp., and *Tanytarsus* sp., as evidenced by increasingly higher electivity indices for these invertebrates. Any changes caused by zebra mussels that positively affect these invertebrates should benefit adult yellow perch in lakes.

More zooplankton was found in the diet of NZM yellow perch in June ($P = 0.005$), and this appeared to be true for the whole year as well. This feeding behavior could have been the result of higher densities of zooplankton in NZM enclosures and (or) the result of compensatory feeding due to a low benthic biomass. We did not measure zooplankton in enclosures with yellow perch, nor did we include zooplankton in the determination of electivity indices, so we do not know if yellow perch were selectively feeding on zooplankton; however, we found more zooplankton in NZM enclosures, suggesting zebra mussels sufficiently depleted algal concentration to lower production of zooplankton. The implications of a lower zooplankton biomass on adult yellow perch is unclear; however, as ZM yellow perch had higher growth rates than NZM yellow perch, this suggests they were capable of compensating for lower zooplanktonic food resources through increased utilization of zoobenthos.

Yellow perch without zebra mussels tended to have more snails in their stomachs in August; however, we found no other evidence from diet analyses or visual observations to support the conclusion that zebra mussels have any substantial effect on snails. The biomass of zoobenthos between treatments with and without yellow perch did not differ, except for a tendency for leech biomass to be lower in yellow perch treatments, indicating yellow perch were adept at feeding on this particular invertebrate group.

Yellow perch with zebra mussels had a positive growth rate while yellow perch without zebra mussels simply maintained their length and lost mass. The difference in growth rate between treatments was highly significant ($P < 0.001$) and was influenced neither by gender nor by enclosure location.

We believe diet is the most likely cause of the observed growth differences between treatments. Sampling yellow perch diet once per month at one time during the day made it difficult to detect diet differences, as yellow perch could have been feeding asynchronously. In fact, we found that yellow perch with similar stomach biomass often showed very different growth responses (e.g., two yellow perch with very different growth rates both had empty stomachs one month). Highly preferred prey (e.g., crustaceans) can be cropped so that its contribution to diet and growth may appear to be negligible in any particular monthly sample. Furthermore, invertebrates that are less frequent in the diet at the time of sampling are more difficult to compare between treatments because of the number of stomachs that are assigned a "0" for that invertebrate. The effect zebra mussels have on an invertebrate would have to be very strong for highly significant differences between ZM and NZM treatments to occur. These difficulties associated with the imperfect methods for fish diet analysis may be compounded

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by a changing benthic community over the summer, making it especially difficult to find significant treatment effects on diet, both temporally and by taxon. For these reasons, we find the results of zoobenthos and diet analyses to be informative despite the lack of strong differences between treatments.

Mackie (1991) predicted Lake St. Clair will become a more oligotrophic lake in which overall productivity (including fishes) will be reduced. Griffiths (1993) has suggested that overall productivity in Lake St. Clair may be reduced if the phosphorus biodeposited into sediment by zebra mussels is not quickly recycled back into the food web. Karnaukhov and Karnaukhov (1993) formulated a model based on data from the former USSR that predicts a severe decline in fish populations in North American waters (where zebra mussel predators are minimal) within 10 years after the invasion of zebra mussels. The results of our study suggest adult yellow perch may not respond in a way that is consistent with these predictions.

Even though yellow perch growth rates in Lake St. Clair are higher than those observed in our pond test fish (Synnestvedt 1997), and despite certain limnological differences between the lake and the pond, yellow perch in zebra mussel infested Lake St. Clair are likely responding to zebra mussels similarly as the pond test fish. In 1994, amphipods were the main dietary component of yellow perch from the lake in May through August, reaching a maximum of 73.6 ± 59.40 amphipods per stomach (males) and 62.9 ± 57.09 amphipods per stomach (females) in August (Synnestvedt 1997). High numbers of amphipods in yellow perch diet can be attributed to zebra mussels. Lake St. Clair contains other benthic prey not found in the study pond that may also benefit from zebra mussels and contribute to higher growth rate of adult yellow perch. Large numbers of the burrowing mayfly *Hexagenia* and crayfish eggs were found in the diet of Lake St. Clair yellow perch in June 1994. The presence of substantial numbers of crayfish eggs in the yellow perch diet may be indicative of an increase in the population of *Orconectes propinquus*, which feeds on zebra mussels (MacIsaac 1994). It is not known at this time if yellow perch in Lake St. Clair grow faster and larger now than they did prior to the zebra mussel invasion. Data from spring trap-net surveys in western Lake Erie in 1992 indicate mass of these yellow perch has increased slightly during the past 5 years (Thomas and Haas 1994); however, conditions existing in Lake St. Clair that are not very prevalent in Lake Erie could affect yellow perch productivity in Lake St. Clair. Lake St. Clair is a much clearer aquatic habitat than years prior to the invasion of zebra mussels. Certain sight-feeding fish may increase in numbers and impose predatory pressure on yellow perch. The authors have observed higher numbers of northern pike (*Esox lucius*), muskellunge (*Esox masquinongy*) and large centrarchids in Lake St. Clair since the invasion of zebra mussels; however, increased water clarity leading to more extensive macrophyte stands will provide yellow perch with more refuge from these predators. In addition, more macrophytes will likely provide more habitat for macroinvertebrates, increasing the prey available to adult yellow perch.

Our study provides the first experimental evidence of how zebra mussels, via an indirect trophic link, affect the productivity of adult yellow perch in North American waters. We have demonstrated that, under relatively rigorous conditions, the higher growth response of adult yellow perch in an experimental pond is causally related to the presence of zebra mussels. Nonetheless, larval yellow perch may respond in entirely different ways than adults, because they are planktivorous. If zebra mussels reduce plankton biomass, larval yellow perch may have difficulty finding food, a limitation that may have population consequences. If zebra mussels simply affect the size distribution of zooplankton, as suggested by MacIsaac et al. (1992), who have hypothesized that only small zooplankters (e.g., rotifers) are being reduced by zebra mussels and that larger zooplankters may not be affected, larval yellow perch may not be severely impacted, since they preferentially feed on larger zooplankters. Continuous monitoring of zebra mussel infested lakes and further experimentation focusing on determinants of fish growth would provide a better understanding of interactions between fish populations and zebra mussels.

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Correlations between pathological changes and chemical contamination in American eels, *Anguilla rostrata*, from the St. Lawrence River

C.M. Couillard, P.V. Hodson, and M. Castonguay

Abstract: American eel (*Anguilla rostrata*) from the St. Lawrence River are heavily contaminated with chemicals that may be associated with increased incidence of diseases and reproductive impairment. The relationship between tissue mirex concentration and body mass was used to separate eels into two groups: the proportion of eels migrating from contaminated areas (Lake Ontario and upper St. Lawrence River) increased as the migration season progressed. Vertebral malformations and basophilic foci in the liver (preneoplastic lesions) were more frequent at the end of the migratory season, when the eels were more heavily contaminated with organochlorine compounds. In contrast, mesenteric nematodes were more common in the first week of the season, when eels were less contaminated. Diameters and percentages of different stages of oocytes, and density and surface area of pigmented macrophage aggregates in the spleen, did not vary among weeks. While basophilic foci are specific biomarkers of exposure to environmental contaminants, vertebral malformations may be caused by a variety of other anthropogenic or natural factors. Further studies are needed to confirm the observed associations between chemical contamination and pathological changes.

Résumé : Les anguilles d'Amérique (*Anguilla rostrata)* du bassin versant du fleuve Saint-Laurent sont fortement contaminées avec des composés chimiques persistants possiblement associés avec une hausse des morbidités et des perturbations dans la reproduction. La relation entre les concentrations tissulaires de mirex et le poids corporel a été utilisée pour séparer les anguilles en deux groupes: la proportion d'anguilles provenant de sites contaminés (Lac Ontario et partie amont du fleuve Saint-Laurent) augmentait à mesure que la saison migratoire avançait. Les malformations vertébrales et les foyers basophiles dans le foie (lésions précancéreuses détectées histologiquement) étaient plus fréquentes à la fin de la saison migratoire, lorsque les anguilles étaient plus fortement contaminées avec des composés organochlorés. À l'opposé, des nématodes mésentériques étaient plus fréquemment rencontrés dans la première semaine de la saison, lorsque les anguilles étaient moins contaminées. Les diamètres et pourcentages d'ovocytes à différents stades de maturation, et les densités et surfaces des agrégats de macrophages pigmentés dans la rate ne variaient pas entre les semaines. Alors que les foyers basophiles sont des marqueurs spécifiques d'une exposition à des contaminants environnementaux, les malformations vertébrales peuvent être causées par une variété d'autres facteurs anthropiques ou naturels. Les associations observées entre contamination chimique et changements pathologiques devront être confirmées par des études ultérieures.

Introduction

Several hundred tons of American eel (*Anguilla rostrata*) are fished each fall in the St. Lawrence Estuary, Québec, Canada, during their seaward migration to spawning grounds in the Sargasso Sea. Most eels captured in the Estuary grow for 12–16 years to sexual maturity in the upper St. Lawrence River and Lake Ontario, highly industrialized areas where they accumulate high loads of persistent contaminants (Hodson et al. 1994). A drastic decline (two orders of magnitude) in the number of juvenile eels migrating upstream to Lake Ontario from the Estuary was observed between 1985 and 1992 (Castonguay et al. 1994*a*). Accumulation of contaminants and toxicity may have contributed to this recruitment failure, although other causes such as oceanographic changes, habitat disturbances, and overfishing are alternative hypotheses (Castonguay et al. 1994*a*, 1994*b*). This study describes pathological lesions and ovarian maturation in migrating American eels and evaluates if there are correlations between chemical contamination and pathological changes.

American eel are benthic, long lived, lipid rich, and reproduce only once in their lifetime, after which they die. In polluted waters, they are likely to accumulate high concentrations of hydrophobic and persistent compounds and to suffer from increased incidence of disease or from reproductive impairment related to toxicity, as do other fish species in the Great Lakes (Baumann and Whittle 1988; Larsson et al. 1991; Mac et al. 1993). In Lake Ontario, several species of benthic fish have developed cutaneous or hepatic tumors associated with exposure to chemicals (Hayes et al. 1990). Significant relationships have been found between concentration of polychlorinated biphenyls (PCB) in eggs and adults and embryonic mortality in Great Lakes lake trout (*Salvelinus namaycush*) (Mac et al. 1993).

Little is known about the health status of American eel in the St. Lawrence drainage basin. Mass mortalities of eels with gill damage and impaired osmoregulation were observed in the

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C.M. Couillard,[1] P.V. Hodson,[2] and M. Castonguay. Department of Fisheries and Oceans, Maurice Lamontagne Institute, 850 Route de la Mer, Mont-Joli, QC G5H 3Z4, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: couillardc@dfo.mpo.gc.ca
[2] Present address: School of Environment Studies, Queen's University, Kingston, ON K7L 3N6, Canada.

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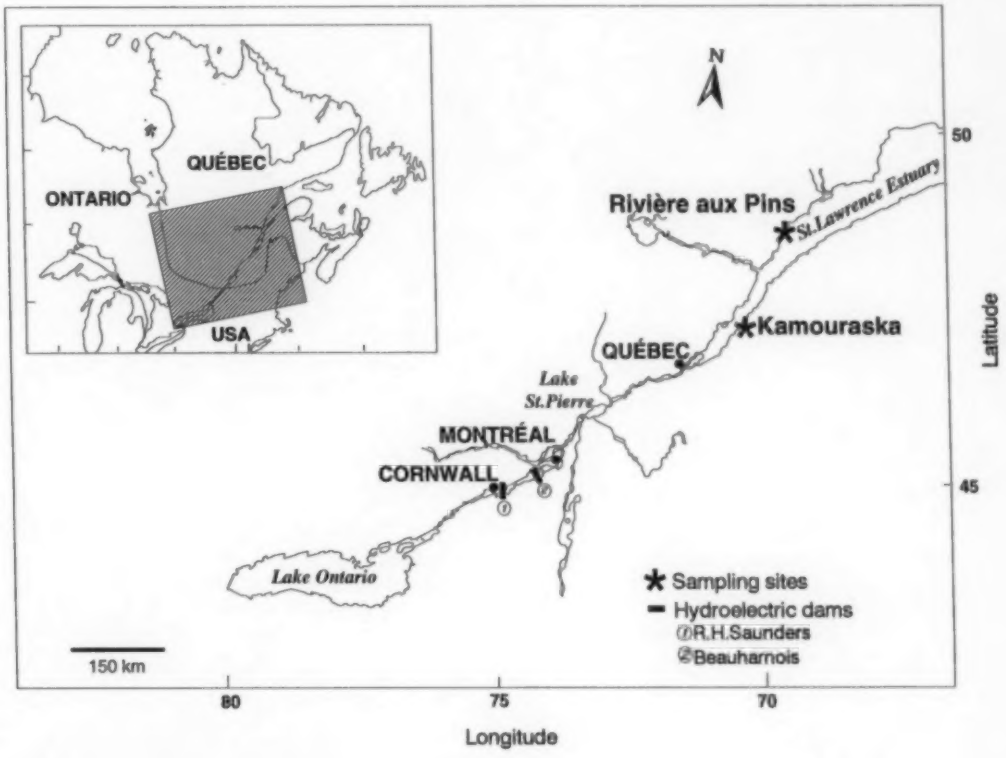
Fig. 1. Map of the study area. Two hydroelectric dams located at Cornwall and at Beauharnois cannot be avoided by American eels migrating from Lake Ontario. Migrating eels were sampled in the St. Lawrence River at Kamouraska and in Rivière aux Pins, a small tributary to the St. Lawrence.



1970s in migrating maturing eels (so-called silver eels) from Lake St-Pierre (river, freshwater) to Kamouraska (estuary, saltwater) (Fig. 1) (Dutil 1984). The cause of these mortalities was not identified, although common bacterial agents were excluded (Dutil and Lallier 1984) and acute toxicity of industrial effluents was suspected (Dutil et al. 1987). Other lesions previously reported include granulomatous parasitic lesions in the gastrointestinal tracts of eels captured in various tributaries of the St. Lawrence River (Cousineau et al. 1977) and vertebral deformities in adults captured by fishermen (Homer 1986). Oocyte diameter increases as silver eels migrate from Lake St-Pierre to Kamouraska but the potential effect of contamination on gonad maturation has never been investigated (Dutil et al. 1985).

In 1990, tissue concentrations of PCB, mirex, and other chlorinated pesticides were 10–100 times higher in silver eels captured at Kamouraska, in the St. Lawrence Estuary than in eels from a reference tributary (Hodson et al. 1994). Samples collected weekly during the 7-week fishing season at Kamouraska (Fig. 1) demonstrated that concentrations of several organochlorine (OC) contaminants increased by more than eightfold with time. This heterogeneous spatiotemporal distribution of contamination gave us an opportunity to determine if levels of contamination were associated with frequencies of gross or microscopic lesions or impairment of oocyte maturation in migrating eels.

The following null hypotheses were tested in migratory maturing American eels sampled weekly at Kamouraska: (*i*) there are no temporal changes in the prevalences of pathological lesions and parasites during the fishing season; (*ii*) prevalences of pathological lesions and parasites are not associated with the level of contamination of the eels; (*iii*) diameter and percentage of vitellogenic oocytes are unchanged in the most contaminated eels; and (*iv*) prevalences of parasites and pathological lesions do not differ between eels from Kamouraska and eels from a reference tributary.

Materials and methods

Sample collection

Maturing female silver eels were trapped in 1990 by a commercial fisherman during their seaward spawning migration using weirs installed at angles to the shoreline at Kamouraska, on the south shore of the St. Lawrence Estuary (Fig. 1). Live eels were purchased weekly during the entire 7 weeks of the fishery, between September 19 and November 1: 100 eels (or less, if fewer eels had been caught) were randomly selected shortly after the fisherman had emptied his traps. Migrating silver eels were also captured in Rivière aux Pins by experimental trap nets on one occasion in the first week of the fishing season. Rivière aux Pins is a reference stream on the north shore of the St. Lawrence Estuary; it drains a forested watershed with no industrial source of chemical contaminants (Fig. 1).

Fish were transported live to the laboratory and held in flowing salt water for 6–48 h before pathological examination. They were anesthetized with MS-222 (tricaine methanesulfonate) and examined

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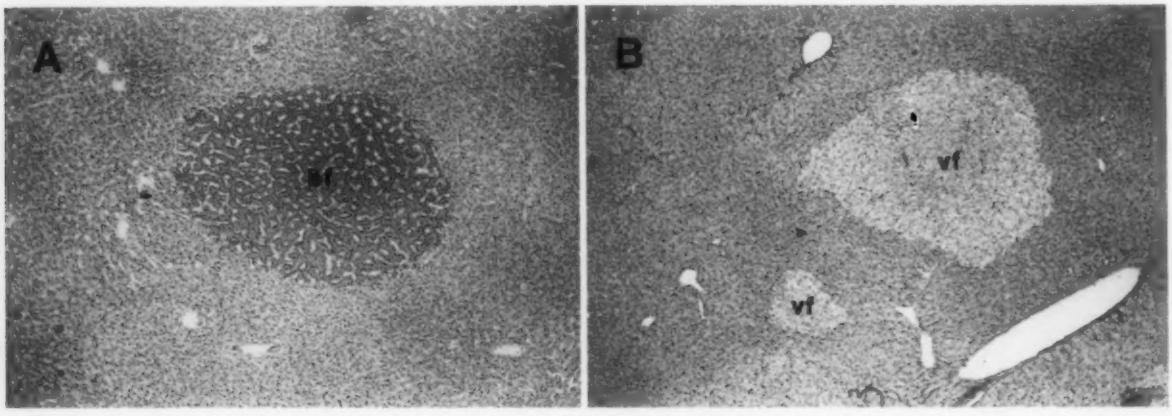
Fig. 2. Foci of hepatocellular alteration in the liver of migrating American eels captured at Kamouraska. (A) Basophilic foci (bf). Hepatocellular cytoplasm is hyperbasophilic and markedly less vacuolated than adjacent hepatocytes. The adjacent tissue is not compressed. Hematoxylin and eosin. 40×. (B) Vacuolated cell foci (vf). Well-delimited zones where the hepatocyte cytoplasm is more vacuolated than that of most hepatocytes. Hematoxylin and eosin. 40×.



for external and internal pathological lesions and parasites. Histological samples were taken from the first 15 eels examined in each sample. Gonads were removed and weighed, and the gutted carcass was frozen for subsequent chemical analyses. Concentrations of PCB, OC pesticides, polycyclic aromatic hydrocarbons (PAH), and mercury were measured in subsamples of 7–16 eels per sample, stratified according to body mass (Hodson et al. 1994). In Kamouraska, only 29 of the 86 eels analysed chemically were also examined histologically but all were examined macroscopically.

Macroscopic pathological examination

We looked for the following lesions previously associated with pollution in fish: vertebral malformations, fin damage, cutaneous ulcers, and cutaneous and liver tumors (Mix 1986; Malins et al. 1988). Prevalences of vertebral deformities, fin damage, and cutaneous lesions were assessed by external examination of each eel. Internal pathological examination was also conducted, and prevalences of nodules in the liver were recorded. A thorough parasitological examination was not performed on each fish, but the presence or absence of commonly occurring and easily- recognized parasites was noted. Presence or absence of the digenea *Azygia longa* in the stomach and of nematodes (species not identified) in the mesentery was recorded.

Histological examination

Liver, gonad, and spleen were excised and fixed in 10% phosphate-buffered formalin. Gonad sections were taken in the middle of the left gonad, and liver sections were taken in the area adjacent to the gall bladder. Tissues were embedded in paraffin, and sections 5 μm thick were stained with hematoxylin and eosin (Luna 1968).

Histopathological examination also focused on the following markers of toxicity: foci of hepatocellular cellular alteration in the liver (preneoplastic lesions) and pigmented macrophage aggregates (PMA) in the spleen (Hinton et al. 1992). In the liver, presence or absence of foci of basophilic hepatocytes was recorded. Basophilic foci are zones of hepatocytes with hyperbasophilic cytoplasm, markedly less vacuolated than adjacent hepatocytes (Fig. 2). These foci, most frequently oval and occasionally with an irregular contour, were not encapsulated and did not compress adjacent tissue. Presence or absence of foci of vacuolation (one or more well delimited zones where the hepatocyte cytoplasm is more vacuolated than that of most hepatocytes) was also determined (Fig. 2).

Pigmented macrophage aggregates (PMA) are focal accumulations of macrophages that may contain four types of brown pigments: melanin, lipofuscin, ceroid, and hemosiderin. Proliferation of PMA has been associated with several natural factors such as aging, starvation, infectious diseases, and parasite infestation (Wolke 1992). Increased density of PMA has also been induced experimentally in fish treated with various toxic compounds and has been observed repeatedly in fish exposed to environmental contaminants (Wolke 1992; Khan et al. 1994; Couillard and Hodson 1996). Density of PMA was evaluated in the spleen by counting the number of PMA at 100× in three randomly selected microscopic fields and dividing this number by the area of the fields examined (2.66 mm^2). The surface area of each PMA counted was measured with an image analysis system (Bioquant, R&M Biometrics®), and mean surface area of PMA was calculated for each eel. PMA were composed of three or more macrophages containing yellow to dark brown pigments within their cytoplasm.

Maturity of oocytes was graded from 1 to 4 with the following morphological criteria: stage 1, no cytoplasmic vacuoles; stage 2, small number of large cytoplasmic vacuoles; stage 3, large cytoplasmic vacuoles forming a complete circle in peripheral cytoplasm and covering less than 50% of the cytoplasm; and stage 4, large and small vacuoles covering more than 50% of the cytoplasm. These criteria have been developed for this study and are based on descriptions of ovarian maturation in European eel (*Anguilla anguilla*) (Colombo et al. 1984). In each gonad section, the proportions of the different stages of oocytes were determined by examining 200 oocytes with a microscope at 160×. The diameters (greatest length through the nucleus) of 10 oocytes of each stage were measured at 160× with the image analysis system.

Statistical analyses

Temporal variation at Kamouraska

Body mass (W), length (L), condition factor ($CF = (W\ (g)/L^3\ (cm)] \times 100$), gonadosomatic index, ($GSI = [\text{gonad mass (g)}/(W\ (g) - \text{gonad mass (g)}]) \times 100$, density and surface area of PMA in the spleen, and percentage and diameter of different stages of oocytes were compared among weeks of capture with the Kruskal–Wallis test, followed, when significant, by analysis of variance on ranked data with Tukey's studentized range test (Conover 1980). GSI was based on "gonad-free body mass" to avoid bias due to autocorrelation and the effects of possible variations among fish in the degree of maturity. Prevalences of vertebral deformities, parasites, and of basophilic and vacuolated foci in liver were compared among weeks of capture with

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Fisher's exact test (Sokal and Rohlf 1981). Confidence intervals (95%) were determined from a table presented in Scherrer (1984) (Table VIII: confidence intervals for small samples). To evaluate the possible effect of lesions on fish condition and to characterize the fish affected with lesions, body mass, length, CF, and GSI were compared between fish with and without vertebral deformities on week 6 and between fish with and without basophilic foci on week 5 of the fishing season. Weeks 5 and 6 were selected on the basis of maximal prevalences of these lesions.

For each week, linear relationships of gonad mass versus somatic mass (body mass – gonad mass) were assessed by least-squares regression. Analysis of covariance (ANCOVA) was used to compare slopes and intercepts of the lines among weeks. The relationship between fish size and gonadal maturation was assessed by comparing the logarithm of stage 4 oocyte diameters (the largest and most abundant oocytes) to the log of body length. The linear model was assessed by least-squares regression for each week and for all weeks combined. The linear relationship between log(density of PMA) and log(body length) was assessed for each week and for all weeks combined by least-squares regression.

Association with contaminants

To test the association between chemical contamination and pathological changes, two approaches were used. First, for the entire fishing season, prevalences of lesions were compared between eels containing high concentrations of mirex, a pesticide concentrated in the upper and most contaminated part of the St. Lawrence basin (Castonguay et al. 1989), and eels with low concentrations of mirex. Second, a dose–response approach was used among variables assessed weekly: prevalences of pathological changes were correlated with concentrations of chemical contaminants and with percentages of eels heavily contaminated with mirex. However, we did not intend to demonstrate cause–effect relationships here, because numerous confounding variables are associated with mirex contamination.

In migrating eels captured at Kamouraska, a linear relationship between log-transformed mirex concentration and body mass was used to discriminate between eels migrating from contaminated areas (Lake Ontario and upper St. Lawrence River) and eels migrating from less contaminated areas. Previous studies have demonstrated the potential use of mirex as a marker of geographic origin in American eels (Dutil et al. 1985; Castonguay et al. 1989). Because tissue concentrations of OC contaminants are frequently related to fish mass (Borgmann and Whittle 1990; Larsson et al. 1991) as a surrogate for age and lifetime contaminant exposure, plotting concentration of mirex as a function of body mass provides a more accurate means of discriminating geographic origin of migrating eels than unadjusted tissue concentrations of mirex alone. Body mass, length, and prevalences of vertebral deformities, parasites, and basophilic and vacuolated cell foci in liver were compared between mirex-contaminated and less contaminated groups of eels by Kruskal–Wallis and Fisher's exact tests. The linear relationships of percentage of contaminated eels versus prevalences of nematode infection, vertebral lesions, and basophilic foci in liver (parameters showing temporal variations) were assessed by least-squares regression.

Concentrations of mirex, heptachlor epoxide, hexachlorobenzene, endrin, and PCB congener 28 varied significantly among weeks (Hodson et al. 1994). The geometric mean concentrations of these contaminants and total PCB in the subsample of eels analysed chemically were correlated with weekly prevalences of pathological lesions in the whole sample. This procedure assumed that the concentrations of contaminants measured in the subsample of eels analysed chemically were proportional to those in the eels examined for gross and histological lesions. This may not be true because the eels examined pathologically were randomly selected; in contrast, the eels examined chemically were selected to represent the range of masses, not the average mass. Log(body mass) and log(body length) were compared by analysis of variance (ANOVA) between fish analysed chemically and the entire sample of eels. Body mass and length were higher in fish analysed chemically on weeks 4 and 5 of the fishing season (data not shown) indicating that, for these weeks, the subsample of eels analysed chemically may not represent the entire sample of eels. The linear relationships of geometric mean concentration of contaminants versus prevalences of nematodes, vertebral lesions, and basophilic foci were assessed by least-squares regression.

Comparison with the reference tributary

Prevalences of pathological lesions and parasites were not compared statistically between sites because the single sample of eels from Rivière aux Pins was not comparable with the multiple samples covering the complete fishing season in the St. Lawrence Estuary at Kamouraska. In contrast, body mass, length, CF, and the density and areas of PMA in the spleen were compared between these sites because these parameters did not exhibit temporal variations in eels from Kamouraska; comparisons were done with the Kruskal–Wallis test. For the first week of the fishing season only, linear relationships between gonad mass and somatic mass were compared between sites with ANCOVA. Diameters and percentages of oocyte stages were compared between sites with the Kruskal–Wallis test.

Results

Temporal variations at Kamouraska

Characteristics of the whole sample and gonadal maturation

Body mass, length, and condition factor (data not shown) did not vary significantly over time of capture (Fig. 3). All eels sampled ($n = 473$) were mature females with well-developed gonads, as expected for the fall fishery in the St. Lawrence Estuary (Dutil et al. 1985).

The GSI tended to increase with time (Fig. 3). ANCOVA indicated that slopes of the relationship between gonad mass and somatic mass did not differ among weeks but that the intercept was highest on week 5 and lowest on week 1 of the fishing season (Table 1). Diameter of stage 2 oocytes was lower on week 7 compared to week 1 (Fig. 4). Diameters of stages 1, 3 or 4 oocytes and percentages of stage 1, 2, 3 or 4 oocytes did not vary significantly among weeks (Fig. 4). During the entire fishing season, percentages of stage 4 oocytes increased slightly as the percentage of stage 2 oocytes decreased. In the last 2 weeks of the fishing season, however, the ratio of stage 4 versus stage 2 oocytes tended to decrease. Diameters of stage 4 oocytes were not correlated to body length for all weeks combined or taken separately.

Macroscopic lesions and parasites

During the entire fishing season, nine eels with vertebral malformations (9 of 473 = 1.9%) were observed. Two types of vertebral malformations were seen: kyphosis (dorsoventral deviation of the spinal column, humpback), most frequently in the area of the neck (six of nine), and scoliosis (lateral deviation of the spinal column), most frequently in the area of the tail (three of nine). Two-thirds of the malformed eels were observed in the last 2 weeks of the fishing season, particularly in week 6 when 10% of eels were affected (Fig. 5). On week 6, body mass, length, CF, and GSI did not differ between eels with or without malformations (Fig. 3). Radiographic examination revealed that 19% of the eels with no external vertebral malformations had a variety of radiographic vertebral lesions (fusions, compressions, dislocations, deformities) (P.V. Hodson,

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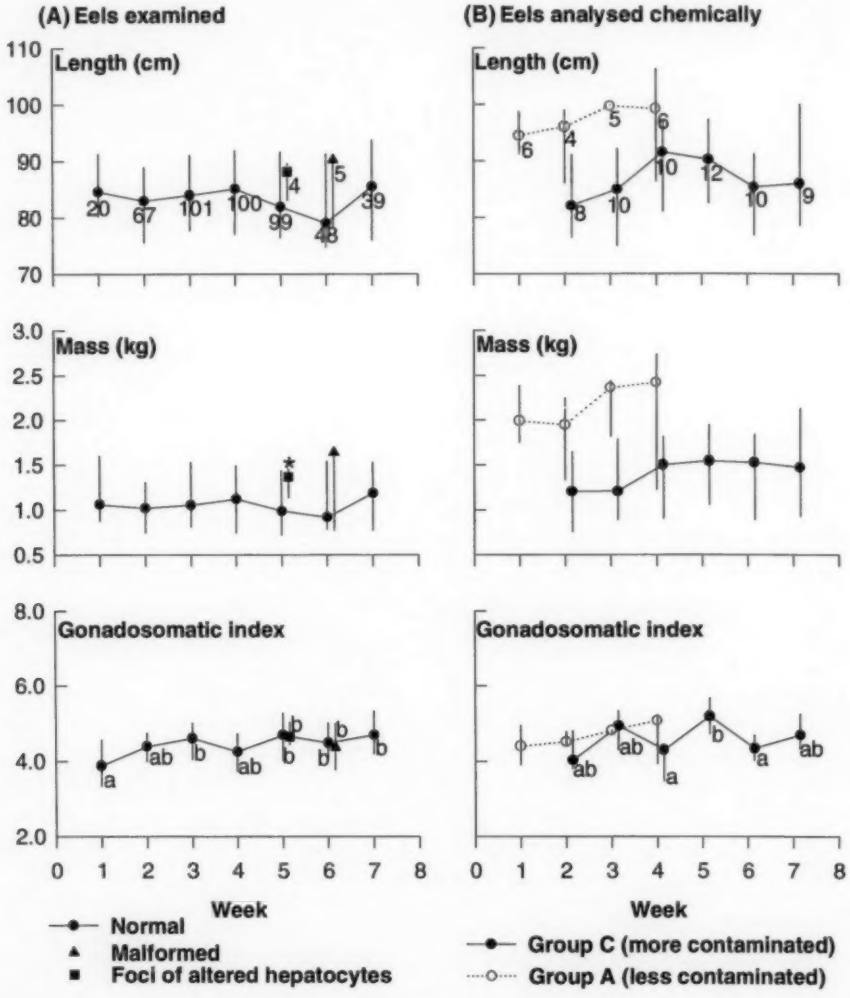
Fig. 3. (A) Temporal variation of body length (*L*), body mass (*W*), and gonadosomatic index (GSI) during the 7-week fishing season in Kamouraska. Medians are given for *L*, *W*, and GSI (bars are from the first to third quartiles). GSI with different letters differ significantly (Kruskal–Wallis, $p \leq 0.05$). Numbers of eels examined for macroscopic lesions are indicated. Characteristics of eels were compared between eels with or without basophilic foci in the liver on week 5 and between eels with and without vertebral malformations on week 6. An asterisk indicates a difference between eels with and without lesions (Wilcoxon, $p \leq 0.05$). (B) Temporal variation of body length, body mass and GSI in eels with high or low mirex contamination during the 7-week fishing season in Kamouraska. Numbers of eels analysed chemically are indicated.



unpublished data). There was no obvious temporal pattern of variation of these radiographic vertebral lesions.

Seven eels (1.5%) had an incomplete caudal fin, with no significant variation in prevalence with time. Other lesions such as cutaneous ulcers, broken eyes, malocclusion of the jaws, abdominal masses, and nodules and cysts in the liver were observed at a prevalence lower than 1% with no significant variation in time (data not shown). Cutaneous wounds were observed in many eels, but their prevalence was not recorded because it was difficult to differentiate between the wounds present before capture from those induced by capture and handling.

Prevalence of mesenteric nematodes was significantly higher in the first week of the fishing season but that of the digenean trematode *Azygia longa* did not vary significantly with time (Fig. 5).

Histological changes

Basophilic foci were observed in the liver of 15 (14%) of the 105 eels examined histologically. A peak prevalence was observed on week 5 (27%) but there was no significant difference among weeks (Fig. 5). On week 5, body mass (Fig. 3) and CF were higher in eels with basophilic foci, while length and GSI did not differ between eels with and without basophilic foci.

Vacuolated foci were observed in 17 eels with a maximal prevalence on week 3, but no significant variation with time of capture (Fig. 5).

Density or surface area of PMA in the spleen did not vary

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Fig. 4. Temporal variation in the diameters and the percentages of different stages of oocytes during the 7-week fishing season in Kamouraska. Stages of maturation of oocytes are graded from 1 to 4 according to morphological criteria. Diameters and percentages are given as means (with SD given in parentheses). An asterisk indicates a difference compared with the first week of sampling (Kruskal–Wallis, $p \leq 0.05$).

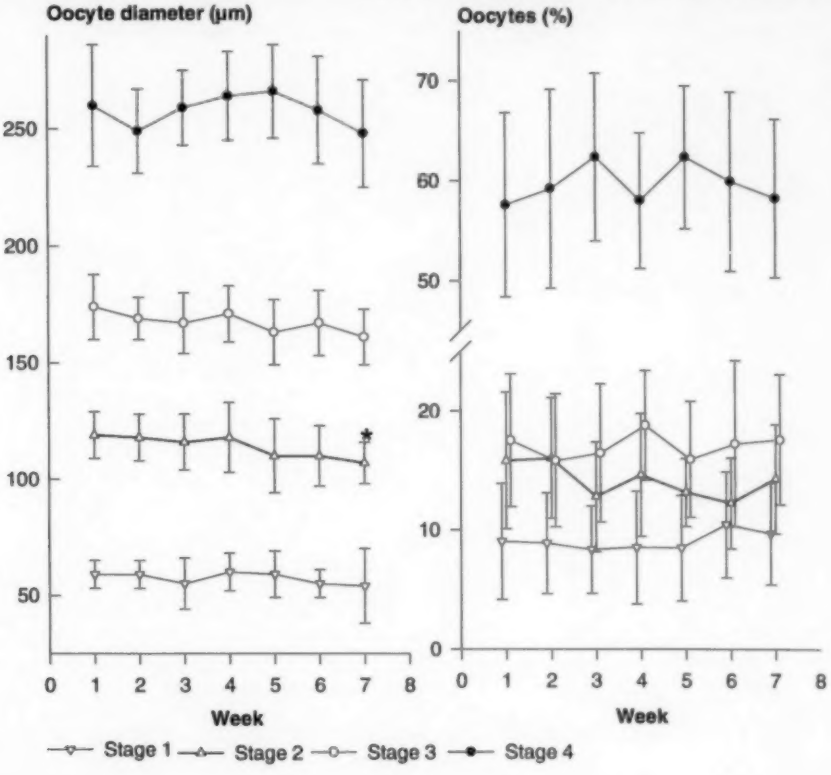

Table 1. Analysis of covariance of gonad mass as a function of somatic mass and time in American eels captured during the 7-week fishing season in Kamouraska.

Week	*n*	Intercept[a]
1	20	−14.8*a*
2	67	−7.6*bc*
3	100	−5.6*cd*
4	99	−10.1*ab*
5	98	−3.8*d*
6	48	−7.1*bcd*
7	39	−5.1*cd*

[a]Gonad mass (g) = 0.05(somatic mass (g)) + intercept. Intercepts with a different letter are significantly different among weeks (ANCOVA, $r^2 = 0.86$, $F = 417$, $p = 0.0001$). Slopes are homogeneous among weeks.

from week to week (Fig. 6). Regressions between density of PMA and body length were not significant for all weeks combined or taken separately.

Association with contaminants

The linear relationship between mirex concentration and body mass successfully separated eels into two main groups: group A, less contaminated ($\log(\text{mirex}) = 0.000\,32\ W - 3.16$, $r^2 = 0.32$, $p = 0.003$, $F = 10.90$, $n = 25$) and group C, more contaminated ($\log(\text{mirex}) = 0.000\,71\ W - 2.17$, $r^2 = 0.55$, $p = 0.0001$, $F = 72.5$, $n = 62$) (Fig. 7). The proportion of group C eels increased with time from 30% in the first week of the fishing season to 75–100% in the last 3 weeks (Fig. 5). Group C eels had lower body mass, length, and CF compared with group A eels (less contaminated) (Fig. 3, Table 2). Group A eels had no vertebral malformations while 6.6% of the group C eels were affected (Table 2). Mesenteric nematodes were found in group A eels but not in group C eels (Table 2). Prevalences of basophilic foci and vacuolated foci were about two times higher in group C than in group A (Table 2). Group C eels also contained higher levels of several other OCs, such as PCB, compared with group A eels (Table 2).

Prevalences of basophilic foci increased, prevalences of nematodes decreased, and the prevalence of vertebral malformations did not vary with the percent of contaminated eels (Table 3). Prevalence of vertebral malformations increased with the concentrations of mirex, endrin, and heptachlor epoxide (Table 3). However, the linear regression was strongly influenced by one data point, a peak of prevalence of malformation on week 6 (Fig. 5). Prevalence of mesenteric nematodes decreased with the concentration of PCB. There was no significant linear relationship between mean concentration of contaminants and prevalence of basophilic foci (Table 3).

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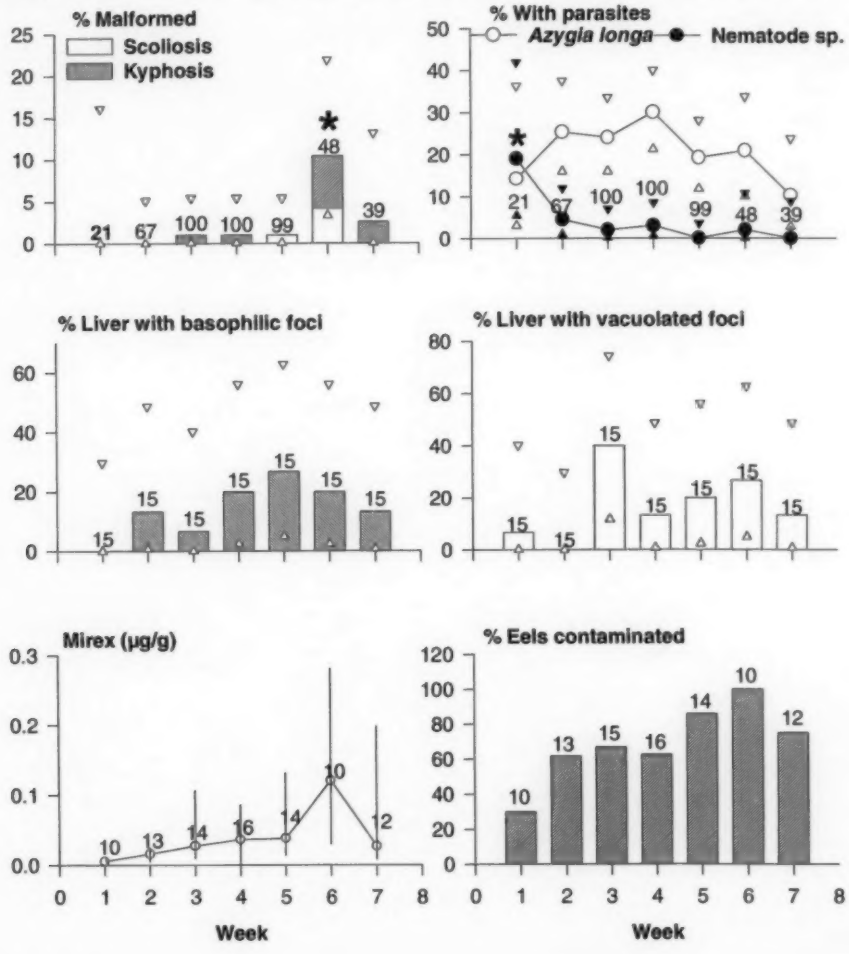
Fig. 5. Temporal variations of prevalence (%) of vertebral lesions, parasites, and histological liver lesions and of the contamination with mirex of eels sampled in Kamouraska. Asterisks indicate a significant variation among weeks (Fisher's exact test, $p \leq 0.05$), triangles indicate 95% confidence intervals. The numbers of eels examined macroscopically (malformations and parasites), histologically (liver lesions), and analysed chemically are indicated. Contamination with mirex is illustrated by median concentrations in carcass (first to third quartiles) and by percentages of eels heavily contaminated with mirex (group C). The numbers of eels analysed chemically are indicated.



Comparison with the reference tributary

Eels sampled at Rivière aux Pins had a lower body mass, length, and CF compared with eels sampled at Kamouraska (Table 4). No vertebral malformations and no nematodes were observed in eels sampled at Rivière aux Pins. Prevalences of the digenean trematode *Azygia longa* was about two times lower in eels from Rivière aux Pins compared with eels from Kamouraska. No basophilic or vacuolated foci were found in the liver of the 15 eels from Rivière aux Pins examined histologically (Table 4). However, with a sample size of only 15, it is only possible to detect diseases with a prevalence higher than 18% at a confidence level of 95% (Martin et al. 1987). PMA were more densely distributed and of smaller size in eels from Rivière aux Pins compared with eels from Kamouraska (Kruskal–Wallis, $p \leq 0.05$) (Fig. 6). The intercept of the linear relationship of gonad mass versus somatic mass was lower in Rivière aux Pins compared with Kamouraska (–9.9 compared with –3.4, ANCOVA, $p \leq 0.05$), and the slopes did not differ between sites. On week 1, diameters of stages 2, 3, and 4 oocytes were lower in Rivière aux Pins compared to Kamouraska (Table 5). Percentages of stage 1 oocytes were higher, and percentages of stage 3 oocytes were lower in Rivière aux Pins (Table 5).

Discussion

The first two null hypotheses were rejected. The prevalences of pathological lesions and parasites (*i*) changed temporally in migrating American eels during the 7-week fishing season at Kamouraska and (*ii*) were associated with the level of contamination of the eels. Vertebral malformations and liver basophilic foci were more frequent at the end of the migratory period, when eels were more heavily contaminated with OCs. In contrast, nematodes were more commonly found in the

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Fig. 6. Density and surface area of pigmented macrophage aggregates (PMA) in the spleen of eels sampled during the 7-week fishing season in Kamouraska and in the first week of the fishing season at Rivière aux Pins. Density and surface area are expressed as median (first to third quartiles).

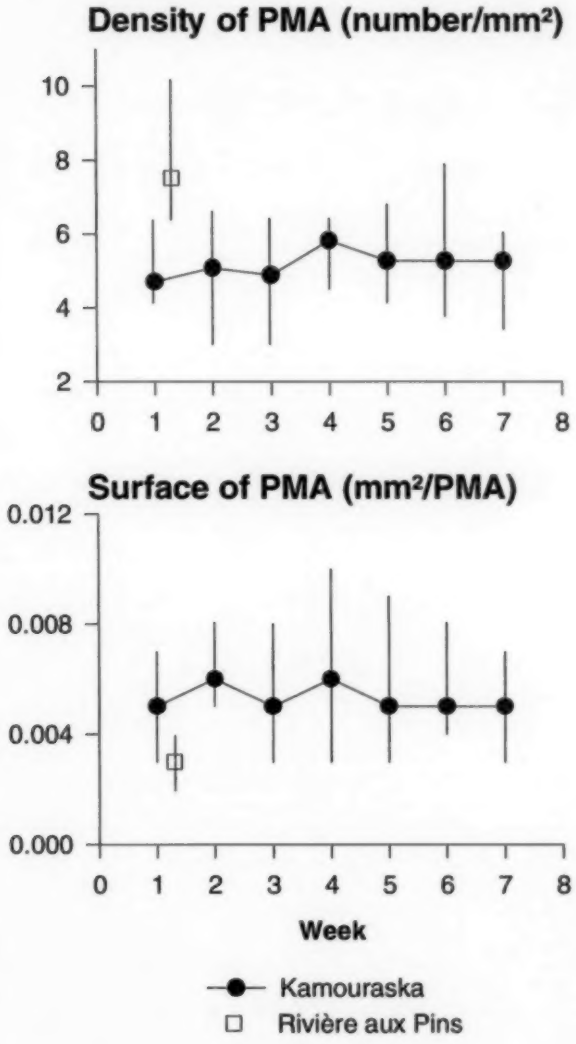
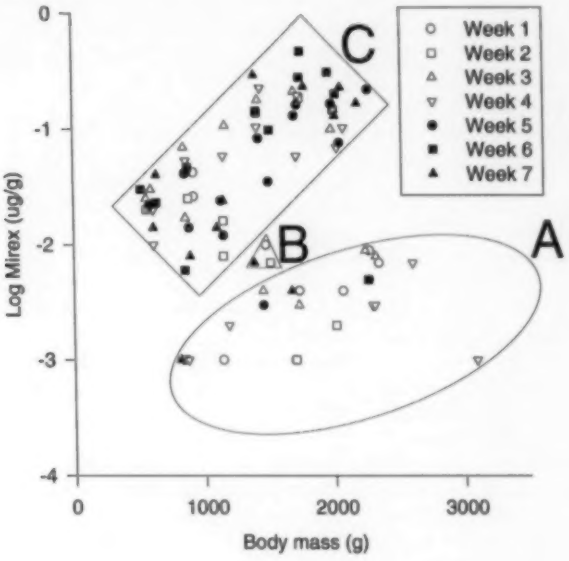


Fig. 7. Concentrations of mirex relative to body mass in eels captured during the 7-week fishing season in Kamouraska, in the St. Lawrence Estuary. Linear relationships between log(mirex concentration) in the carcass and log(body mass) are used to discriminate between eels migrating from contaminated areas (group C) and eels migrating from less contaminated areas (group A).



abdomen of eels captured in the first week of migration, when the eels were less contaminated. Density of splenic PMA did not vary with time. The third null hypothesis was accepted: (*iii*) histological evaluation of the gonads did not reveal any disturbance of ovarian maturation; diameters and percentages of different stages of oocytes did not vary among weeks. Finally, (*iv*) eels sampled in the reference tributary, Rivière aux Pins, had no vertebral malformations, had no basophilic nor vacuolated foci in the liver, and had low prevalences of parasites compared with eels sampled in Kamouraska. However, it was not possible to statistically compare the prevalences of lesions between the single sample at Rivière aux Pins and the multiple samples covering the whole fishing season in the St. Lawrence River, at Kamouraska.

In fish, vertebral malformations may be a consequence of fractures of vertebrae, vitamin deficiencies, altered embryonic development, parasitic infections, electric shock, or exposure to environmental contaminants (Claveau 1989; Hinton 1993). Prevalences of vertebral malformations have been positively correlated with the levels of several OCs and these compounds (and (or) associated contaminants) are one possible cause of the observed malformations (Couch et al. 1977; Mehrle et al. 1981; Bengtsson et al. 1985). However, eels migrating from their most contaminated habitat, Lake Ontario, also go through the turbines of two large hydroelectric dams or their associated ship locks (Fig. 1), that could cause vertebral fractures and spinal deformities, especially in larger eels (Larinier and Dartiguelongue 1989). About 20% (16–24%) of the silver eels injected experimentally in the turbines of the Beauharnois hydrolectric dam died with cutaneous wounds and fractures of the vertebral column (Desrochers 1995). Survivors had no external lesions, but long-term survival and radiographic lesions were not evaluated. In 1992, 12 000 eels captured commercially in five areas of the St. Lawrence River basin including Lake Ontario and Kamouraska were examined for external lesions. Vertebral malformations were about six times more frequent in migrating eels from Kamouraska compared with yellow (resident) eels from Lake Ontario (Dutil 1994; Dutil et al. 1997). Migrating eels were larger and probably older than resident eels, so that the effects of age, size, and migration could not be distinguished. Nutritional deficiencies or contaminant effects on bone metabolism would probably cause

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defects in other bones or cartilage, but this was not observed (Hinton 1993). *Myxosoma cerebralis*, a parasite commonly associated with vertebral deformities (Markiw and Wolf 1978), was not found in two malformed eels examined (data not shown). The two leading hypotheses for the causes of vertebral malformations, toxicity and (or) physical damage by the hydroelectric dams, should be tested experimentally and by field investigations. Prevalences of radiographic vertebral lesions should be evaluated in resident eels, at different contaminated sites in the Lake Ontario – St. Lawrence River axis, and in migrating eels as they pass through the R.H. Saunders and Beauharnois hydroelectric dams (Fig. 1).

The arrival of malformed eels in the last weeks of migration could indicate that they have locomotory impairment and slowed migration. Alternatively, eels originating from the most contaminated areas such as Lake Ontario may arrive late because they have a longer distance to cover or a delayed onset of migration. Therefore, while both contaminant concentrations and the frequency of vertebral malformation increase late in the fishing season, no firm conclusion can be reached regarding associations between the two. Contaminants might contribute to vertebral damage, or both responses may simply be indicators of the origin of the eels and the route travelled during the seaward migration.

Hepatic basophilic foci have been described in various fishes exposed experimentally to chemical carcinogens and in wild fish exposed to industrial and urban pollution; they are considered specific biomarkers of exposure to environmental contaminants (Hinton et al. 1992). In rodents and fish, this lesion represents a preneoplastic stage in the histogenesis of hepatocellular neoplasms (Hinton et al. 1988). Basophilic foci, adenomas, and hepatocellular carcinoma have been described in white sucker (*Catostomus commersoni*) captured in Hamilton Harbour, a site on the western shore of Lake Ontario heavily contaminated with PAH and metals (Hayes et al. 1990). Hepatocellular carcinomas have also been induced experimentally in rainbow trout (*Oncorhynchus mykiss*) injected with an extract of sediment from the same site (Metcalfe et al. 1990). In our study, prevalences of basophilic foci were not correlated with concentrations of individual OCs but rather with the proportion of heavily contaminated eels. Other contaminants, such as PAH, may be responsible for the lesions. This is the first report of foci of hepatocellular alteration in American eels, and the prevalences of these lesions and of possibly related liver tumors in resident eels are not known.

Basophilic foci are benign lesions that are not likely to interfere with normal migration, as indicated by the normal CF and gonad development of affected eels. However, the number of eels examined histologically was too small to detect hepatocellular carcinomas and adenomas, and the prevalence and severity of lesions may be underestimated if eels severely affected by carcinomas and adenomas do not migrate. Tumors and preneoplastic lesions may also be more frequent in older eels, and weekly variations in age may influence the variation in prevalences of basophilic foci. Unfortunately, we could not age eels in this study, because there is no published reliable aging method for American eels from the St. Lawrence River from bony structure (Castonguay et al. 1994*a*). Correlations of contamination and pathological changes would also have been more informative if all analyses (chemical, pathological, and histological) had been conducted on the same individuals.

Table 2. Body size and prevalences of pathological changes and parasites in two groups of American eels captured at Kamouraska, characterized by low or high concentration of mirex.

Variables	Less contaminated (group A)	More contaminated (group C)
Length (cm)	97.8 (91.2–99.7)	86.5 (78.6–95.0)*
Body mass (g)	2110 (1533–2418)	1471 (904–1838)*
CF (g/cm^3)	0.23 (0.20–0.24)	0.20 (0.19–0.22)*
n	25	61
Prevalences of lesions and parasites (sample size)		
Vertebral malformations	0 (25)	6.6 (61)
Mesenteric nematodes	20 (25)	0 (61)**
Azygia longa	20 (25)	16 (61)
Basophilic foci in liver	9 (11)	22 (18)
Vacuolated foci in liver	9 (11)	17 (18)
Contaminants		
Mirex (μg/g)	0.003 (0.001–0.005)	0.064 (0.023–0.162)**
PCB (μg/g)	0.25 (0.19–0.43)	0.95 (0.42–1.30)**

Note: Body mass (W), length (L), condition factor (CF = (W (g)/L^3 (cm)) × 100), and tissue concentrations of total PCB congeners are given as medians (with the first to third quartiles given in parentheses).

*Significantly different from group A (Wilcoxon, $p \leq 0.05$).

**Significantly different from group A (Fisher's exact test, $p \leq 0.05$).

Vacuolated foci may appear in the liver during the process of gonadal maturation because of vitellogenesis and transfer of fatty material from the liver to the gonads (Timashova 1981). Vacuolated foci could also be phenotypically altered hepatocytes, also known as clear cell foci, that are frequently associated with basophilic foci in fish exposed to carcinogens (Hinton et al. 1992). It is difficult to differentiate these two types of histological changes in maturing fish. Histochemical markers may be useful in demonstrating altered enzymatic activity within preneoplastic foci (Teh and Hinton 1993).

Density of PMA did not vary temporally as a function of the degree of contamination and did not differ between more and less contaminated eels at Kamouraska. Density of PMA increases with age in several fish species (Wolke 1992). Tagging studies have demonstrated that age may vary from 10 to 20 years in eels migrating from Lake Ontario (Castonguay et al. 1994*a*). It may be necessary to control for this confounding factor to demonstrate a possible effect of contaminants. Density of PMA did not correlate with body length, but length of maturing eels is probably not closely correlated with age (Helfman et al. 1987; Bouillon and Haedrich 1996). Density of PMA was higher in eels captured at Rivière aux Pins, which may begin migration at an older age compared with eels captured at Kamouraska. Factors governing the onset of sexual maturation are not well known in American eels; age and size at maturity appears to vary greatly with latitude and with different habitat characteristics (Helfman et al. 1987; Bouillon and Haedrich 1996). Starvation associated with migration may be another factor affecting the density of PMA in eels since tissue catabolism causes an increased size and (or) number of splenic PMA (Agius and Roberts 1981; Wolke 1992). Without parallel information on individual eel age and feeding status,

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Table 3. Regression analysis of the prevalences of pathological changes as a function of the concentration of various contaminants or of the percentage of eels highly contaminated with mirex during the fishing season at Kamouraska ($n = 7$, $y = ax + b$).

	Vertebral malformations					Mesenteric nematodes					Basophilic foci in liver				
	a	*b*	r^2	*F*	*p*	*a*	*b*	r^2	*F*	*p*	*a*	*b*	r^2	*F*	*p*
Pesticides															
Mirex	7.6	14.6	0.59	7.1	0.05	–14.8	–19.6	0.62	8.1	0.04	15.5	39.4	0.41	3.5	0.12
Endrin	10.2	26.5	0.57	6.6	0.05	–8.5	–15.8	0.11	0.6	0.47	14.4	48.5	0.19	1.2	0.33
Heptachlor epoxide	22.0	45.3	0.80	19.9	0.007	–22.9	–40.3	0.23	1.6	0.26	27.4	67.8	0.21	1.3	0.30
Sum of PCB congeners	14.6	5.9	0.34	2.6	0.17	–41.0	–5.8	0.74	14.6	0.01	34.3	22.9	0.32	2.3	0.19
% contaminated eels	0.1	–6.0	0.52	5.4	0.07	–0.3	22.7	0.68	10.9	0.02	0.32	–7.6	0.61	7.8	0.04

Table 4. Body size and prevalences of lesions and parasites in American eels captured at Rivière aux Pins and at Kamouraska.

Variables	Rivière aux Pins (week 1)	Kamouraska (weeks 1–7)
Body length (cm)	60 (56–69)	84 (77–92)*
Body mass (g)	368 (369–570)	1172 (852–1670)*
CF (g/cm^3)	0.17 (0.16–0.19)	0.20 (0.18–0.22)*
n	100	474
Prevalences of lesions and parasites (sample size)		
Vertebral malformations	0 (100)	1.9 (474)
Mesenteric nematodes	0 (100)	2.7 (474)
Azygia longa	9 (100)	23 (474)
Basophilic foci in liver	0 (15)	14 (105)
Vacuolated foci in liver	0 (15)	17 (105)
Contaminants		
Mirex (µg/g)	0.001 (0.001–0.001)	0.024 (0.007–0.107)*
PCB (µg/g)	0.007 (0.005–0.008)	0.54 (0.32–1.10)*
n	7	90

Note: Body mass (W), length (L), and condition factor (CF = [W (g)/L^3 (cm)] × 100) are given as medians (with the first to third quartiles given in parentheses).

*Significantly different from Rivière aux Pins (Wilcoxon, $p \leq 0.05$).

Table 5. Mean (with SD given in parentheses) diameters and percentages of different stages of oocytes in American eels collected in Kamouraska and Rivière aux Pins on the first week of the fishing season.

	Kamouraska	Rivière aux Pins
Diameter (µm)		
Stage 1	59 (6)	55 (6)
Stage 2	119 (10)	106 (11)*
Stage 3	174 (14)	152 (13)*
Stage 4	260 (26)	235 (19)*
Percentages (%)		
Stage 1	9 (5)	14 (6)*
Stage 2	16 (6)	13 (5)
Stage 3	18 (6)	13 (3)*
Stage 4	58 (9)	61 (8)

Note: Fifteen eels were analysed at each sampling location.

*Significantly different from Kamouraska (Wilcoxon, $p \leq 0.01$).

splenic PMA density in eels cannot be used as a reliable biomarker of environmental contamination.

Mesenteric nematodes were encountered only in less contaminated eels captured at Kamouraska and not in more contaminated eels from Kamouraska or in uncontaminated eels from the reference tributary, Rivière aux Pins. Thus, these parasites are probably indicative of the origin of the eels and not of chemical contamination. They could potentially be used to discriminate geographic origins of migrating eels (Williams et al. 1992), but further detailed parasitological studies would be needed to fully evaluate this method of discrimination.

It was not possible to discriminate the freshwater origin of the eels sampled at Kamouraska on the basis of the presence or absence of mirex (Hodson et al. 1994), but the relationship between mirex concentration and body mass provided a very clear separation. We were able to demonstrate that percentages of eels with higher loads of mirex increased with time during the fishing period and that, for the entire fishing season, 71% of the eels probably came from the Lake Ontario – St. Lawrence River axis, assuming Lake Ontario is the primary source. On the basis of presence or absence of mirex, Dutil et al. (1985) estimated that 74% of migrating eels captured between Kamouraska and Lake St-Pierre in 1982 came from Lake Ontario. Between 1982 and 1990, levels of mirex in eel flesh have decreased by 56%, and mirex contamination has spread downstream from its source in Lake Ontario, probably via riverine and atmospheric transport (Castonguay et al. 1989; Sergeant et al. 1993; Hodson et al. 1994); eels from the St. Lawrence Estuary tributaries are now contaminated with low levels of mirex (Hodson et al. 1994).

All gonad sections showed a large proportion of vitellogenic oocytes with diameters within the range of those reported previously for migrating silver American eels (Wenner and Musick 1974). We expected oocyte maturation to progress throughout the migration season, but oocyte diameters and proportions of various stages did not vary significantly among weeks. These parameters were very variable within weeks and larger sample sizes may be necessary to document temporal variations.

The health of any migrating animal population is difficult to evaluate because of complex and variable patterns of spatial and temporal distributions of different cohorts. Intensive daily sampling throughout the migration period may be required to obtain reliable estimates of lesion prevalences (Dutil 1994). However, despite a limited weekly sampling effort, the prevalences of external lesions estimated in this study are comparable with those estimated in eels captured at Kamouraska in 1992 with an intensive daily sampling schedule (Dutil 1994; Dutil et al. 1996). Prevalences of lesions in the migrating population may have been underestimated because diseased eels may die before reaching the Estuary or may be less vulnerable to traps. The apparent good health (low prevalences of

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lesions) of the highly contaminated eels captured in Kamouraska may be a consequence of this sampling bias and may not be a reliable index of the health of the whole population.

This study demonstrates an association between contamination and pathological changes in American eel. As a descriptive study, it was not intended to demonstrate cause–effect relationships, because numerous confounding variables are possibly associated with OC contamination. For example, eels that are more heavily contaminated with OCs may also be older, have a longer distance to travel, be exposed to more migratory stresses, pass through the turbines of hydroelectric dams, and be exposed to other contaminants or to habitat deterioration (Couillard et al. 1992). All these factors must be assessed, as covariates, together with OC contamination, to understand potential causes for the observed changes. Our objective was to identify health problems possibly related to contaminants and to indicate priorities for further studies on the health status of American eel in the St. Lawrence basin. These research priorities include studies of (*i*) effects of contaminants on prevalences of vertebral malformations and preneoplastic and neoplastic lesions in resident eels in Lake Ontario and other highly contaminated habitats and (*ii*) effects of hydroelectric dams on long-term survival and vertebral malformations in migrating silver eels. The possible impacts of contaminants on eel reproduction should also be assessed by investigating effects of contaminants (iii) on steroid hormone levels and metabolism and (*iv*) on developing embryos of American eel. These studies are important because both pathological changes and reproductive disturbances may have a deleterious impact on the migratory and reproductive success of the declining population of American eels.

Migrating American eels have been proposed as a vector of contaminants for beluga whales (*Delphinapterus leucas*) and may be a source of specific toxic compound that are present in Lake Ontario but not in the St. Lawrence Estuary (Lum et al. 1987; Hickie et al. 1991; Béland et al. 1993; Hodson et al. 1994; Muir et al. 1996). In the St. Lawrence Estuary, American eels and beluga whales have the following common characteristics: both species are highly contaminated with OCs including mirex originating from Lake Ontario and the upper St. Lawrence River, both have experienced low population sizes and finally, and both have preneoplastic or neoplastic lesions suggesting exposure to environmental carcinogens (this study; Martineau et al. 1994). For both species, it is difficult to establish the link between contamination and the observed health problems because of sampling bias and of multiple factors other than contaminants possibly affecting the population. Additional studies are needed to further our understanding of effects of eel contamination on the health and reproduction of eels and belugas.

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Taxon diversity in the genus *Holopedium* (Crustacea: Cladocera) from the lakes of eastern North America

Paul D.N. Hebert and Terrie L. Finston

Abstract: Although the genus *Holopedium* contains only two recognized species, the taxonomic status of many North American populations has been uncertain. By exploiting allozyme analysis to clarify taxonomic assignments, this study shows that *Holopedium* populations in the lakes of eastern North America are separated into two genetically distinct lineages. Component populations of each group show both low levels of genetic polymorphism and high genetic similarity. Aside from their genetic divergence, morphological and ecological differences among the groups support their recognition as distinct species. *Holopedium gibberum* is broadly distributed in the cool temperate regions of North America, while *H. amazonicum* occurs in the southern and eastern portions of the continent. The latter pair of species co-occur at some sites without hybridization, confirming their reproductive isolation.

Résumé : Bien que le genre *Holopedium* ne compte que deux espèces reconnues, le classement taxinomique de nombreuses populations d'Amérique du Nord demeure incertain. La présente étude démontre, par le recours à l'analyse des allozymes pour clarifier le classement taxinomique, que les populations d'*Holopedium* dans les lacs de l'est de l'Amérique du Nord appartiennent à deux lignées génétiquement distinctes. Les populations constituant chacun des groupes montrent à la fois un faible degré de polymorphisme génétique et une grande ressemblance génétique. Outre leur divergence génétique, les groupes montrent des différences morphologiques et écologiques qui justifient leur reconnaissance comme des espèces distinctes. *Holopedium gibberum* est répandu dans les régions à climat tempéré d'Amérique du Nord, tandis que *H. amazonicum* se rencontre dans les parties sud et est du continent. Ces deux paires d'espèces cohabitent dans certains sites sans qu'il y ait hybridation, ce qui confirme leur isolement du point de vue reproduction.
[Traduit par la Rédaction]

Introduction

Although many species of zooplankton are thought to possess distributions that span two or more continents (Banarescu 1990), critical taxonomic work has suggested that most of these cases involve species complexes (Frey 1982). The difficult taxonomy of many groups of zooplankton appears linked to both their extreme phenotypic plasticity (Jacobs 1961; Dodson 1989) and the prevalence of interspecific hybrids (Wolf and Mort 1986; Taylor and Hebert 1993). Recently, genetic analyses have become an increasingly important component of taxonomic studies on zooplankton. This approach has the advantage of allowing access to characters that are usually unaffected by environmental conditions and that can permit a direct indication of the reproductive status of morphologically divergent forms.

Genetic analyses have now clarified species boundaries in a number of zooplankton groups. The most intense effort has so far been directed towards the genus *Daphnia*. Allozyme studies on the daphniid fauna of southeastern Australia revealed the confusion of at least seven different species as *D. carinata* (Hebert 1977; Hebert and Wilson 1994), while work in North America more than doubled the size of the fauna from 15 to 34 species (Brooks 1957; Hebert 1995). Other zooplankton genera have not been the target of such detailed investigations, but there has been sufficient work to confirm that similar taxonomic problems are of general occurrence. Allozyme analyses on North American members of the cladoceran genus *Bosmina* revealed the presence of twice as many species as previously recognized (DeMelo and Hebert 1994*a*, 1994*b*). Similarly, genetic studies on the calanoid copepod, *Hesperodiaptomus arcticus*, have indicated that it is a complex of at least three species (Boileau 1991). In each case, morphological differences were noted among the newly recognized species subsequent to genetic analysis. Hence, earlier failures to recognize species arose not from the lack of diagnostic characters but rather from difficulties in the a priori selection of these informative characters from the host of other variable traits. These results make it clear that the broader application of genetic analyses is likely to significantly advance our understanding of zooplankton taxonomy.

The present study employs allozyme analysis to investigate the taxonomic diversity of *Holopedium*, a dominant member of the zooplankton fauna in softwater lakes across North America. The genus presently comprises two species (Korovchinsky 1992): *H. gibberum*, described from central Europe, and *H. amazonicum*, described from the Amazon delta. *Holopedium gibberum* is thought to have a broad Holarctic distribution, while the distribution of *H. amazonicum* is unclear because of taxonomic confusion. Analysis of *Holopedium* populations from the southeastern United States revealed populations combining characters diagnostic of

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P.D.N. Hebert[1] and T.L. Finston. Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: phebert@uoguelph.ca

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H. gibberum with those typical of *H. amazonicum*, leading Hegyi (1973) to suggest that these species might represent a single variable taxon. Tessier (1986) later found striking life-history differences between *Holopedium* populations from two lakes in Pennsylvania. Although individuals from these populations possessed the diagnostic features of *H. gibberum*, they showed a twofold difference in both the body size of their primiparous females and in the number of their anal spines. Tessier (1986) suggested that these two forms might represent different species and, from a broader survey, concluded that some North American populations closely resembled *H. gibberum*, while others were morphologically intermediate to this species and *H. amazonicum*. Montevilo et al. (1987) subsequently reported the existence of two types of gelatinous sheaths in *Holopedium* from lakes in eastern North America. Although the divergence in sheath morphology was invariably associated with a second morphological difference, the presence or absence of a basal spine on the anal claw, Montevilo et al. (1987) did not conclude that the forms were different species. The failure of these past morphological studies to clarify species boundaries within the genus provided the incentive for the present investigation.

Prior genetic work on *Holopedium* has been limited to an allozyme survey of 20 populations from lakes in the eastern United States (Thier 1992). This study revealed the occurrence of marked heterozygote deficits in some *Holopedium* populations, a result that was attributed to inbreeding (Thier 1994). However, reports of similar heterozygote deficits in other cladocerans (Smith and Fraser 1976) were later shown to reflect the sympatric occurrence of two or more reproductively isolated species (Hann and Hebert 1982). This study reexamines patterns of genetic diversity in *Holopedium* populations of lakes in eastern North America and provides a limited analysis of morphological diversity among populations.

Materials and methods

Collections

Seventy-one habitats from 17 sites in the temperate regions of eastern North America containing the genus *Holopedium* were sampled between May 1990 and July 1996. The sites and names of lakes or reservoirs from which populations were collected are as follows, prefixed by the state or province name and a centrally located urban center: CANADA: New Brunswick (NB), St. Andrews ((1) Lomond, (2) Utopia, (3) First, (4) Gibson, (5) Skiff, (6) Kerr, (7) North, (8) Chamcook); Newfoundland (NF), Badger ((1) unnamed pond); Ontario (ON), Dorset ((1) 5 Mile, (2) Beech, (3) Brady, (4) Hall, (5) Paint, (6) Prospect, (7) Shoe, (8) Spring, (9) Wren, (10) Clear, (11) Bigwind, (12) Bird, (13) Echo, (14) Grandview, (15) Gullfeather, (16) St. Nora, (17) Wolfkin, (18) Chub, (19) Kushog, (20) McCormick, (21) Fairy, (22) Rill), Timmins ((23) Byng, (24) Radisson); Quebec (QC), St. Donat-de-Montcalm ((1) Archambault, (2) Rond, (3) Beland), Frontenac Park ((4) St. Francois), northern Quebec ((5) St. Jean, (6) Chibougamau); UNITED STATES: Florida (FL), Waldo ((1) Dorr, (2) Santa Fe); Georgia (GA), Chattahoochee National Park ((1) Blue Ridge, (2) Banks); Maine (ME), Farmington ((1) Flying Pond, (2) Crowell, (3) Torsey, (4) Long, (5) Moosehead, (6) Boyd, (7) Hebron, (8) Messalonskee, (9) Maranacook), Machias ((10) Patrick); Michigan (MI), Watersmeet ((1) Thousand Islands); Minnesota (MN), Boundary Waters ((1) Maple, (2) Pughole); North Carolina (NC), Morgantown ((1) James), Smokey Mountain National Park ((2) Santeetlah, (3) Hiwassee); Pennsylvania (PA), Lake Ariel ((1) Corilla, (2) Forest, (3) Lacawac); Wisconsin (WI), Eagle River ((1) Rest, (2) Crystal Bog, (3) Day, (4) Lynx, (5) Mud, (6) North Turtle, (7) Nebish, (8) Merril, (9) Wood). These habitats were, in general, chosen simply to provide representatives of *Holopedium* populations across the study area. However, samples were collected from two (Flying Pond, Messalonskee) of the five Maine lakes where Thier (1992, 1994) detected heterozygote deficits, as well as from one (Lacawac) of the two lakes in Pennsylvania where Tessier (1986) noted life-history variation. While Thier's samples were collected in May and June, our resampling of these lakes occurred in late July. Collections were usually made with a 400 µm mesh tow net, and, when possible, 50 individuals were immediately frozen in liquid nitrogen. As well, individuals were preserved in 70% ethanol for subsequent morphological analysis.

Electrophoresis

Forty-four individuals from each of the habitats (= population) were routinely examined, along with reference standards (individuals from ON6), for variation at nine enzyme loci: arginine kinase (*Apk*); fumarate hydratase (*Fumh*); asparate aminotransferase, mitochondrial and supernatant forms (*Aat-m*, *Aat-s*); malate dehydrogenase, mitochondrial and supernatant forms (*Mdh-m*, *Mdh-s*); mannose phosphate isomerase (*Mpi*); glucose phosphoisomerase (*Gpi*); and phosphoglucomutase (*Pgm*). Cellulose acetate gel electrophoresis permitted the characterization of single individuals for variation at all nine loci. Standard methods were employed (Hebert and Beaton 1993), excepting that individuals were removed from their gelatinous sheaths prior to homogenization. The R_f values were calculated for each allele at a locus in relation to the mobility of the common allele in the reference population. It is important to note that R_f value differences of 3% or less are difficult to detect when gel resolution is poor. To confirm the validity of R_f differences reported in this study, all values represent the mean of several runs.

Genetic characterization of populations

The percentage of polymorphic loci and individual heterozygosities, as measured by direct count, were determined for all populations of each species. As well, allele frequencies were calculated, and the number of unique multilocus genotypes was ascertained for each population. A locus was considered to be polymorphic in a specific population when the frequency of the most common allele did not exceed 0.95. Genotypic frequencies at polymorphic loci were tested for conformance to Hardy–Weinberg (HW) expectations, employing Levene's correction for small sample sizes (Levene 1949).

The genotypic structure of each population was initially summarized using three parameters: the number of unique multilocus genotypes (N), the logarithmic probability of genotypic arrays at polymorphic loci (LOGPROB), and the genotypic diversity ratio (GDR). The methods of calculation and rationale for their use are discussed in Hebert et al. (1988). Briefly, LOGPROB is calculated as an average of the logarithmic probability of the observed genotypic array at each polymorphic locus, so that, for example, a value of –2 indicates that the genotypic arrays at the polymorphic loci in a population have an average probability of 0.01 of being in HW equilibrium. The GDR represents the ratio of the observed number of multilocus genotypes to the number expected in a sample of the same size from a sexually reproducing population with similar allele frequencies. The mean and variance of the expected genotypic diversity for each population was obtained from 100 Monte Carlo simulations. The probability that the observed number of genotypes differed from that expected was assessed using a t test of single observations against the corresponding mean and variance (Sokal and Rohlf 1981). Populations were classified as being sexually reproducing and consisting of a single species when the LOGPROB ranged from 0 to –2.00, and the GDR was not significantly different than expected. Populations were classified as possessing a deviant genotypic composition when the LOGPROB was more negative than –2.00, and the GDR showed

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Fig. 1. Morphological characters used in the discrimination of *Holopedium*.

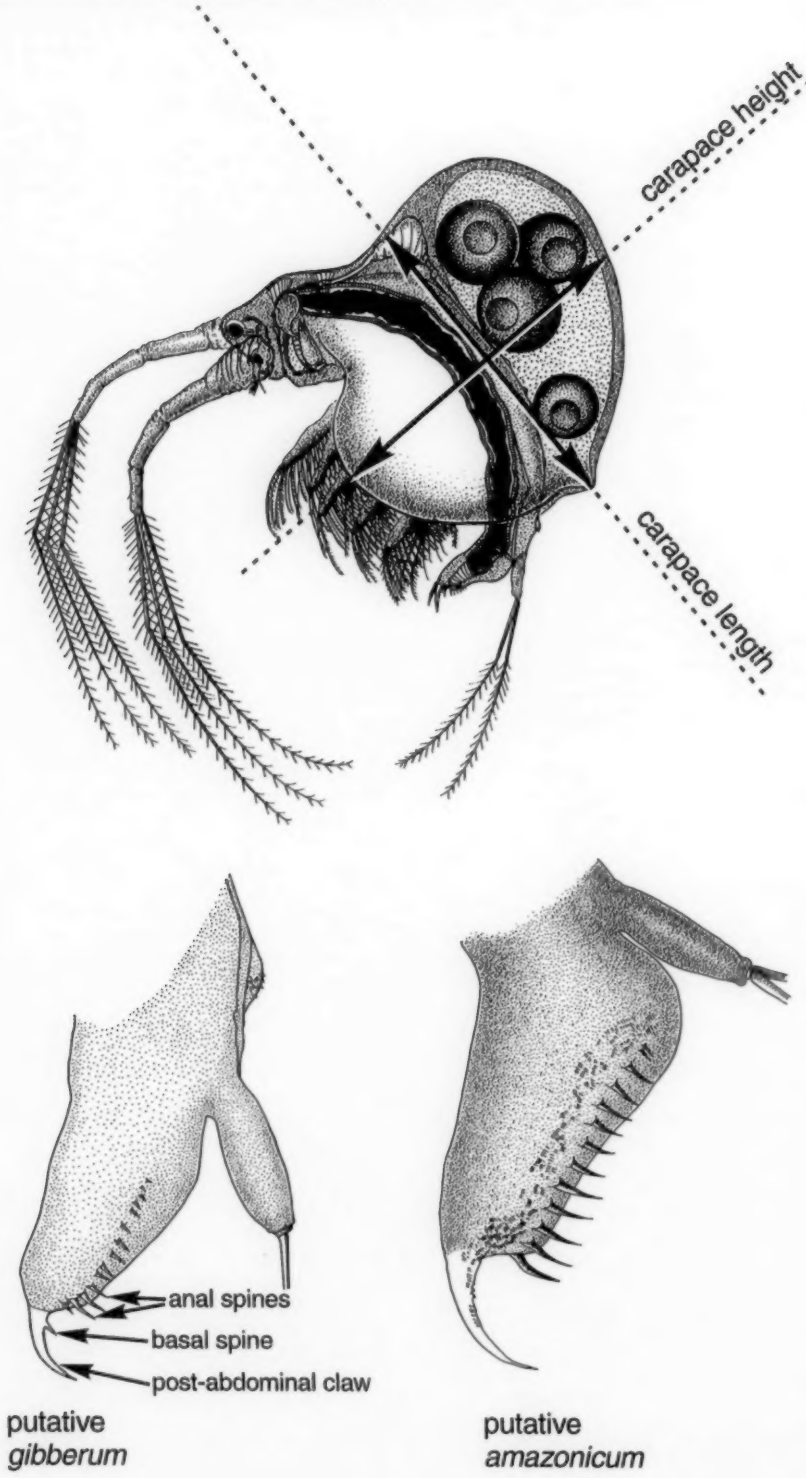

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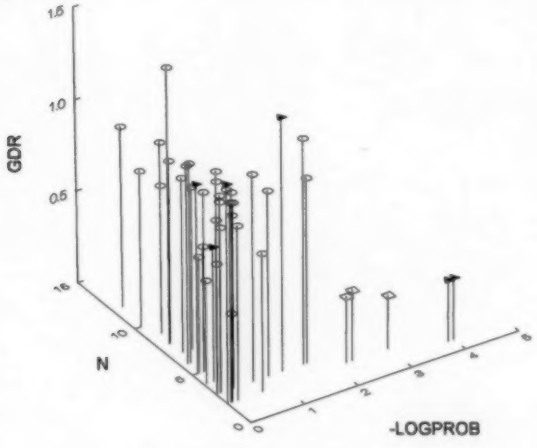
Fig. 2. Genotypic characteristics of 48 polymorphic populations of *Holopedium*. Symbols indicate the taxonomic composition of individual populations based on subsequent UPGMA analysis. Triangles, *H. amazonicum*; circles, *H. gibberum*, squares, mixed populations.



significant deviation from that expected. Such deviations can arise as a consequence of the dominance of populations by interspecific hybrids, by species co-occurrence without hybridization, or by the abandonment of sexual reproduction (Hebert and Wilson 1994).

Roger's genetic distance (1972) was used to quantify the extent of genetic differentiation among single-species populations, and this distance measure was subsequently used to produce a cluster diagram of genetic distances using the UPGMA algorithm in BIOSYS (Swofford and Selander 1989).

Wright's (1978) *F* statistics were calculated to examine levels of genetic differentiation among populations of the two common species at three hierarchical levels. The hierarchy was constructed such that divergence was measured among demes within a state or province (F_{DS}), among demes across the entire survey region (F_{DT}), and among states or provinces across the survey region (F_{ST}).

Morphometrics

Twelve populations were selected for morphometric analysis. When possible, 10 individuals from each population were measured for the following variables: valve length from the compound eye to the posterior tip of the carapace, valve height from ventral to dorsal points on the carapace along the midline, number of anal spines, and number of basal spines on the postabdominal claw (Fig. 1). As bilateral asymmetry in the number of basal spines was occasionally noted, individuals lacking a spine were checked for its presence on the other claw. In cases where a spine was detected on only one claw, the individual was recorded as possessing this trait. In addition, individuals were classified as possessing either a simple gel sheath or one with lateral projections (see Montevilo et al. 1987 for details). Body size measurements were made at 18× using a JAVA image-capture system, while spine counts were done at 100×. Visualization of the gel sheath was aided by staining the whole animal in a dilute solution of toluidine blue.

Summary statistics for all populations were calculated for each variable. Variable means were tested for heterogeneity using a one-way analysis of variance. Tukey's test was used to test individual pairwise comparisons to reveal the source of the variation. A discriminant function analysis was subsequently employed to examine the contribution of each variable to the overall observed variation among taxa. Discriminate function analysis is used when individuals can be allocated a priori to groups and maximizes between-group variation while minimizing within-group variation.

Results

Genetic characterization of populations

Twenty-three of the 71 populations were monomorphic at all 9 loci. Of the 48 polymorphic populations, only 5 showed both a significantly lower GDR than expected and significant HW deviations (Fig. 2). Two of these populations were collected in the southern United States (NC1, NC2), and their deviant genotypic attributes were due to heterozygote excess at a single locus (*Gpi*). By contrast the three other deviant populations from New Brunswick (NB1, NB2, NB3) showed a complete lack of heterozygotes, despite the co-occurrence of two homozygous genotypes. The five deviant populations were temporarily removed from the data set, while the remaining populations, which were either monomorphic at all loci or conformed to HW expectations and had a nonsignificant GDR, were assumed to represent single species. These 66 populations were on average, polymorphic at 9.9% of their loci (range 0–33%), while heterozygosities of individuals averaged 3.2% (range 0–13%).

UPGMA cluster analysis of genetic distances among the 66 single-species populations indicated the occurrence of two genetically divergent groups of populations that are hereafter regarded as distinct species (Fig. 3). *Holopedium amazonicum* included populations from the eastern and southeastern regions of North America, while *H. gibberum* was most common in the central region of the continent but also occurred in the northeast.

Seven of the nine loci were polymorphic within at least one taxon, while two loci, *Mdh-m* and *Aat-m*, were fixed for the same allele in both taxa. The two species showed either marked gene frequency shifts or allele substitutions at six of the seven polymorphic loci (Table 1). *Holopedium gibberum* possessed a unique allele at both *Apk* and *Mdh-s*, which discriminated it from *H. amazonicum*. Retrospective analysis of the five populations with deviant genotypic attributes indicated that both populations from the southern United States possessed allelic arrays typical of *H. amazonicum*. By contrast, the deviant populations from New Brunswick included an admixture of individuals showing genotypic arrays typical of either *H. gibberum* or *H. amazonicum*. No hybrids or unexpected combination of alleles, which would indicate introgression, were detected in these populations. Genetic distances were then recalculated among the *Holopedium* populations from all 71 habitats; gene frequency data for the *Holopedium* from the two southern habitats were entered without manipulation, while those from the three northern habitats were entered after partitioning individuals into two subgroups that showed fixed differences at *Apk* and *Mdh-s*. The UPGMA resulting from analysis of the full data set possessed a topology similar to that of the original analysis (Fig. 3). Five of the populations (NC1, NC2, NB1–NB3) were assigned to *H. amazonicum* and showed close affinities with the NC3 and NB4–NB6 clade. The other three populations (NB1–NB3) were assigned to *H. gibberum* and were genetically identical to NB7.

There was substantial variation in gene frequencies among local populations of both taxa with F_{DS} values of 0.40 and 0.57

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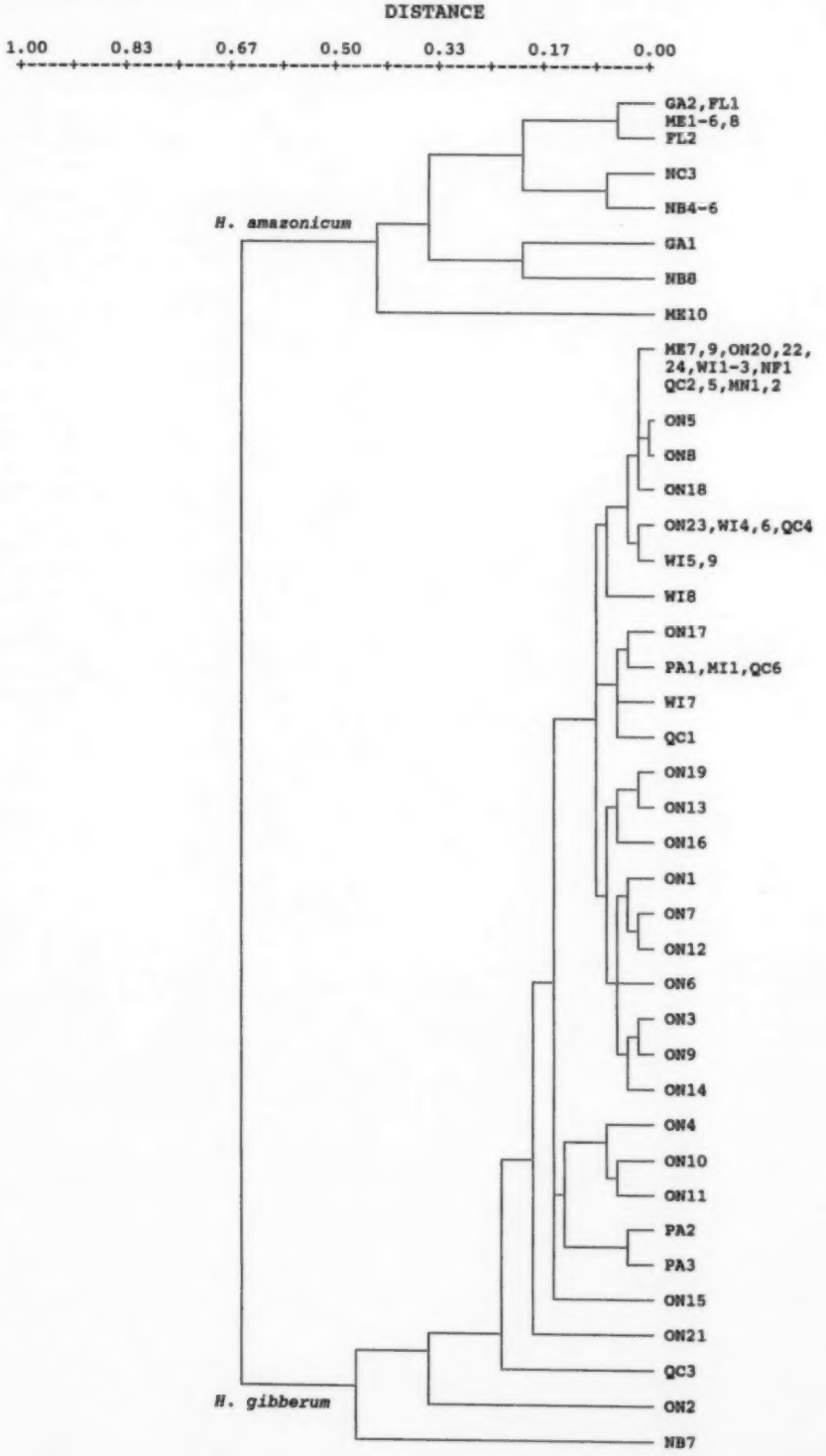
Fig. 3. UPGMA of Rogers genetic distances for 66 single-species populations of. *Holopedium* from eastern North America. Nodes are collapsed for populations showing $D < 0.01$.



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for *H. gibberum* and *H. amazonicum*, respectively (Table 2). *Holopedium gibberum* showed little variation in gene frequencies among aggregate populations from different states or provinces, as evidenced by a F_{ST} of 0.08. Regional patterning of gene frequencies was stronger in *H. amazonicum*, which showed a F_{ST} of 0.33 largely as a result of a north–south shift in gene frequencies at the *Aat-s* locus (Fig. 4).

Morphometrics

Individuals of the two species showed conspicuous morphological differences (Table 3). Hence, *H. gibberum* invariably possessed a jelly coat with a lateral lobe and a basal spine on their postabdominal claw, while individuals of *H. amazonicum* had a smooth jelly coat and lacked a basal spine. Individuals of *H. gibberum* showed great variation in both carapace length and anal spine numbers, but individuals of *H. amazonicum* showed less variation in these traits (Fig. 5).

Joint consideration of carapace length and height as well as the numbers of anal and basal spines using discriminant function analysis showed that individuals of the two species could be reliably discriminated (Fig. 6). The number of basal spines contributed most to the variation along the first discriminant axis, while much of the variation along the second axis was due to carapace length and, to a lesser extent, the number of anal spines.

Discussion

Allozyme analysis showed that 66 of 71 populations of *Holopedium* in temperate eastern North America possessed genotypic arrays similar to those expected in single-species populations. Genetic distance analyses revealed that these populations were separable into two genetically divergent groups, with each group consisting of individuals homozygous at most loci and differing from the other group by allelic substitutions at two or more loci. This pattern of genotypic structure might reflect either the occurrence of two, weakly polymorphic species or the occurrence of inbred lines of a single species. If the latter explanation was valid, one would expect a large number of inbred lines showing, in aggregate, all possible allelic combinations. The present results rule out this possibility, indicating instead the occurrence of two well-differentiated groups of genotypes, showing genetic distances typical of distinct species. Moreover, individuals assigned to these groups showed an obvious divergence in postabdominal claw structure and sheath morphology. Their joint morphological and genetic divergence supports the recognition of each group as a distinct species.

The results of this study are, in a sense, congruent with those of Thier (1994) who found that *Holopedium* from lakes in the northeastern United States split into two genetically divergent groups, as would be expected if his collections included the two species that our study detected from lakes in this area. Although he attributed the heterozygote deficits detected in *Holopedium* populations from some Maine lakes to inbreeding, his two dominant genotypes showed allele substitutions corresponding to those between *H. amazonicum* and *H. gibberum*. Moreover, our reanalysis of lakes in his study area showed that both of these taxa were present in this region. Thier's work did suggest the presence of additional genotypic combinations in three lakes, but our reanalysis of these

Table 1. Gene frequencies at seven variable loci for two species of *Holopedium* from eastern North America.

			Apk		*Gpi*				*Pgm*			*Mpi*			*Mdh-s*		*Fumh*			*Aat-s*		
Taxon	Region	*n*	1.0	1.05	0.97	1.0	1.12	1.21	0.85	0.90	1.0	0.90	0.96	1.0	1.06	1.0	1.13	1.0	1.1?	0.8?	0.91	1.0	1.09
gibberum	NB	1	1.0	—	—	1.0	—	—	—	1.0	—	—	—	1.0	—	1.0	—	1.0	—	—	—	1.0	—
	NF	1	1.0	—	—	1.0	—	—	—	—	1.0	—	—	1.0	—	1.0	—	1.0	—	—	—	1.0	—
	ON	24	1.0	—	—	0.93	0.07	—	0.01	0.21	0.78	—	—	0.54	0.46	1.0	—	0.99	0.01	—	0.01	0.99	—
	QC	6	1.0	—	0.04	0.95	0.01	—	—	0.16	0.84	—	0.01	0.99	—	1.0	—	1.0	—	—	—	1.0	—
	ME	1	1.0	—	—	1.0	—	—	—	1.0	—	—	—	0.94	0.06	1.0	—	1.0	—	—	—	1.0	—
	PA	3	1.0	—	—	1.0	—	—	—	0.71	0.29	—	—	1.0	—	1.0	—	1.0	—	—	—	1.0	—
	MN	2	1.0	—	—	1.0	—	—	—	—	1.0	—	—	1.0	—	1.0	—	1.0	—	—	—	1.0	—
	WI	9	1.0	—	—	1.0	—	—	—	0.18	0.82	—	0.01	0.99	—	1.0	—	1.0	—	—	—	0.96	0.04
	MI	1	1.0	—	—	1.0	—	—	—	0.50	0.50	—	—	1.0	—	1.0	—	1.0	—	—	—	1.0	—
amazonicum	NB	4	—	1.0	—	—	1.0	—	1.0	—	—	—	1.0	—	—	—	1.0	0.83	0.17	—	—	1.0	—
	ME	9	—	1.0	—	—	0.98	0.02	.99	.01	—	0.04	0.96	—	—	—	1.0	1.0	—	—	—	1.0	—
	NC	1	—	1.0	—	0.11	0.89	—	1.0	—	—	—	0.89	0.11	—	—	1.0	1.0	—	—	0.19	0.81	—
	GA	2	—	1.0	—	—	1.0	—	1.0	—	—	—	1.0	—	—	—	1.0	1.0	—	—	0.78	0.22	—
	FL	2	—	1.0	—	—	1.0	—	1.0	—	—	—	1.0	—	—	—	1.0	1.0	—	0.04	0.96	—	—

Note: Allelic frequency dated are pooled for all populations (*n*) from each province or state that were diagnosed to consist of a single species. Alleles are discriminated by their R_f value, with a question mark indicated for rare alleles whose mobility was not accurately determined.

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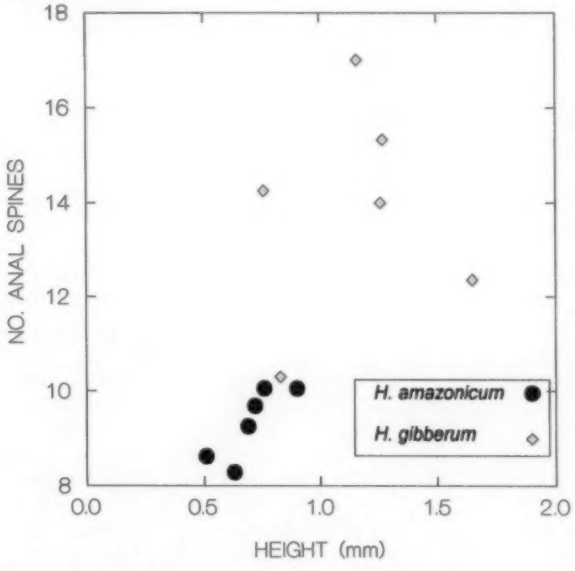
Table 2. Wright's (1978) hierarchical *F* statistics, with variance given in parentheses, for populations of *H.amazonicum* and *H. gibberum*.

	amazonicum			*gibberum*		
Locus	F_{DS}	F_{ST}	F_{DT}	F_{DS}	F_{ST}	F_{DT}
Aat-s	0.64 (0.08)	0.57 (0.16)	0.84 (0.24)	0.81 (0.04)	0.10 (0.01)	0.82 (0.05)
Fumh	0.77 (0.14)	0.06 (0.00)	0.76 (0.06)	—	—	—
Mpi	0.20 (0.01)	0.04 (0.00)	0.17 (0.01)	0.38 (0.10)	0.08 (0.02)	0.43 (0.12)
Gpi	0.17 (0.02)	0.25 (0.03)	0.38 (0.05)	0.32 (0.02)	–0.04 (0.00)	0.29 (0.02)
Pgm	—	—	—	0.38 (0.13)	0.09 (0.03)	0.43 (0.16)
Mean	0.57 (0.24)	0.33 (0.21)	0.72 (0.44)	0.40 (0.29)	0.08 (0.06)	0.44 (0.35)

Note: The *F* statistics are defined as follows: F_{DS}, variation among demes in a state or province; F_{ST}, variation among populations from states or provinces in eastern North America; F_{DT}, variation among demes in eastern North America.

Fig. 4. Allele frequency variation at the *Aat-s* locus in populations of *H. amazonicum*. Data from all single-species populations in each state or province are pooled, even those showing HW disturbances.

Fig. 5. Bivariate plot of characters (number of anal spines, valve height) showing greatest discriminating power among populations of *Holopedium*.



populations suggests that misscoring of phenotypes at the *Mdh-s* locus created these new genotypes.

Our study revealed polymorphisms in populations of both species of *Holopedium* permitting determination of their breeding systems. The close approximation of genotypic frequencies to HW equilibrium and the presence of genotypic diversities similar to those expected in a sexual species indicate that both *H. gibberum* and *H. amazonicum* reproduce by cyclic parthenogenesis. The distributional data so far available suggest that these species have largely allopatric distributions (Fig.7). *Holopedium amazonicum* dominates lakes in eastern Canada and the southern United States, whereas *H. gibberum* seems to have a broader but more northerly distribution in the central regions of North America. Although not broadly sympatric, *H. gibberum* and *H. amazonicum* do co-occur in some lakes in Maine and New Brunswick. Interestingly, the presence of a morphologically diverse *Holopedium* fauna in this region of North America was first recognized nearly 70 years ago (Carpenter 1931). The present study revealed only three cases of sympatry, but our reinterpretation of Thier's (1994) results indicates their co-occurrence in six other lakes. At each of these sites, the species co-existed without hybridization, confirming their reproductive isolation.

The morphological and distributional differences noted between the two species identified in this study explain the patterns of morphological divergence noted in earlier work on North American *Holopedium*. Hegyi's (1973) observation that populations of *Holopedium* from the southeastern United States invariably lack a spine on their postabdominal claw is

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Table 3. Mean, with SD given in parentheses, for four variable morphological characters in populations of *Holopedium* and a qualitative description of the jelly coat.

	Lake	Length (mm)	Height (mm)	Basal spines	Anal spines	Jelly coat
H. gibberum	ME7	0.75 (0.21)	0.76 (0.26)	1.00 (0.00)	14.25 (2.12)	Lobed
	ME9	0.79 (0.10)	0.83 (0.10)	1.00 (0.00)	10.30 (1.16)	Lobed
	ON6	1.12 (0.12)	1.16 (0.23)	1.00 (0.00)	17.00 (2.00)	Lobed
	PA1	0.85 (0.13)	1.27 (0.19)	1.00 (0.00)	15.33 (2.12)	Lobed
	PA3	0.91 (0.15)	1.26 (0.19)	1.00 (0.00)	14.00 (1.83)	Lobed
	WI7	1.26 (0.13)	1.65 (0.15)	1.00 (0.00)	12.36 (2.46)	Lobed
H. amazonicum	FL1	0.61 (0.08)	0.69 (0.09)	0.00 (0.00)	9.30 (0.95)	Smooth
	ME1	0.89 (0.13)	0.90 (0.09)	0.00 (0.00)	10.10 (1.20)	Smooth
	ME2	0.78 (0.06)	0.76 (0.08)	0.00 (0.00)	10.10 (1.29)	Smooth
	NB4	0.55 (0.15)	0.63 (0.08)	0.00 (0.00)	8.33 (1.53)	Smooth
	NB8	0.53 (0.14)	0.51 (0.02)	0.00 (0.00)	8.67 (1.16)	Smooth
	NC3	0.61 (0.14)	0.72 (0.14)	0.00 (0.00)	9.73 (1.35)	Smooth

Fig. 6. The first two discriminant functions of morphological variables measured for 10 individuals for each of 12 populations of *Holopedium*.

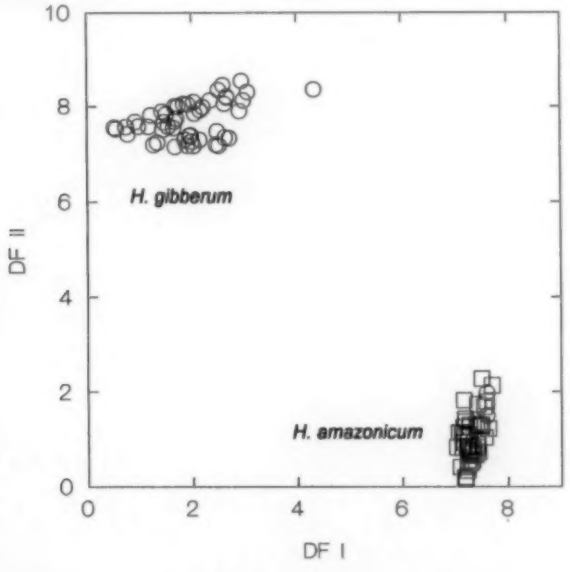

Fig. 7. Pie diagrams showing the proportions of each of two *Holopedium* species at sites in eastern North America.

explained by the dominance of *H. amazonicum* in this area. The discovery by Montevilo et al. (1987) of the linked divergence in sheath morphology and spinescence on the postabdominal claw in populations from the eastern United States is a result of their analysis of both *H. gibberum* and *H. amazonicum*. Some of the morphological and life-history differences noted by Tessier (1986) probably have a similar origin, but his results also suggest either the presence of additional species in the *gibberum* group or its extreme phenotypic plasticity. Indeed, this study has confirmed that populations of *H. gibberum* do show dramatic morphological variability. Interestingly, local populations of both *H. amazonicum* and *H. gibberum* show more genetic differentiation than reported in other species of lake zooplankton (Mort and Wolf 1985; DeMelo and Hebert 1994*b*). This suggests that populations are often founded from few individuals, setting the stage for large shifts at gene loci that control life history and morphology.

The present study contributes to our growing awareness of regional divergence in the species composition of zooplankton communities. In particular, the distinctive *Holopedium* faunas of the eastern and central regions of North America reinforce a pattern detected in other aquatic organisms. Prior work has, for instance, established that the faunal composition of zooplankton communities in eastern Canada is strikingly different from that in other regions of the country. For example, *D. pulex*, which is a dominant element of the zooplankton in ponds across much of Canada, is nearly replaced by *D. minnehaha* in the east (Hebert 1995). As well, populations of widely distributed fish species typically have distinct eastern lineages whose origin has been linked to their occupancy of an Atlantic refugium throughout the Pleistocene (Wilson et al. 1996; Wilson and Hebert 1996).

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This study has again confirmed the value of genetic analyses in driving the taxonomic revision of zooplankton groups. This progress results because the diagnosis of species boundaries, through genetic analysis allows a posteriori morphological appraisals. It is, as a result, possible to identify variable traits that reflect species boundaries as opposed to those that arise from environmental variation. This study sets the stage for a more thorough examination of taxonomic diversity in *Holopedium* at sites across North America. It points, as well, to the need for genetic studies to accompany efforts to ascertain the extent of phenotypic diversity in single zooplankton species.

Acknowledgements

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Late-summer distribution of phytoplankton in relation to water mass characteristics in Hudson Bay and Hudson Strait (Canada)

Michel Harvey, Jean-Claude Therriault, and Nathalie Simard

Abstract: Descriptive and multivariate analytical methods were used to analyze the early September (1993) abundance and species composition of phytoplankton in relation to water mass characteristics in Hudson Bay and Hudson Strait. Four groups of stations distributed along well-defined environmental gradients characterizing the distribution of physical and chemical variables were identified. The first group, located in the most southern region of Hudson Bay, was strongly influenced by freshwater runoffs from James Bay and from the other major rivers around the bay and was characterized by a relatively high phytoplankton biomass (chlorophyll *a* (Chl *a*) > 1.0 $\mu g \cdot L^{-1}$) in the near-surface waters and by a phytoplankton assemblage equally dominated by small flagellates and dinoflagellates. The second group, located in an area northwest of the Belcher and Sleeper islands, was characterized by relatively well-mixed conditions where small diatoms composed about 50% of the phytoplankton assemblage. The third group occupied the upper part of the bay and the entrance of the strait and was characterized by the lowest surface nutrient concentrations encountered. A clear subsurface chlorophyll maximum dominated by small flagellates (>55% of the assemblage) was observed in this region. The fourth group was located in the central part of the strait where the highest surface nutrient concentrations and phytoplankton biomass values (Chl *a* > 2.0 $\mu g \cdot L^{-1}$) were observed. The phytoplankton assemblage there was clearly dominated by small diatoms (>80%). These conditions are related to the presence of more intense tidal mixing in this region. The phytoplankton standing crop within this area was comparable with that observed during an autumn bloom situation in most temperate regions of the world's ocean.

Résumé : Différentes méthodes d'analyses statistiques ont été utilisées afin d'étudier l'influence des facteurs environnementaux sur l'abondance et la composition spécifique du phytoplancton dans les 50 premiers mètres de la colonne d'eau dans la Baie et le Détroit d'Hudson au début de septembre 1993. Ces analyses ont permis de subdiviser l'aire d'étude en quatre régions (ou groupe de stations) distribuées successivement depuis la partie supérieure de la Baie jusque dans la partie centrale du Détroit. Chacune de ces régions est influencée par des processus hydrodynamiques différents affectant la stabilité verticale de la colonne d'eau. La première région se caractérise par une prédominance de dinophycées et de microflagellées et par une biomasse phytoplanctonique relativement élevée (chlorophylle *a* (Chl *a*) > 1.0 $\mu g \cdot L^{-1}$) près de la surface. Le maintien de cette communauté phytoplanctonique résulte de la forte influence des apports d'eau douce en provenance de la Grande rivière de la Baleine et de la baie James. Le second groupe de stations se situe au nord-ouest des îles Belcher et Sleeper dans une région fortement influencée par la gyre cyclonique caractérisant le patron général de circulation des masses d'eau dans la baie d'Hudson. Les effets combinés de ce patron de circulation et de la présence des îles Belcher et Sleeper provoquent un mélange vertical des masses d'eau favorisant le maintien d'une communauté phytoplanctonique dominée par les diatomées (>50 % de l'assemblage). La troisième région s'étend de la partie supérieure de la Baie jusqu'au Détroit d'Hudson. Elle se caractérise par une faible biomasse phytoplanctonique (Chl *a* < 0.05 $\mu g \cdot L^{-1}$), une forte stratification verticale et de faibles apports d'éléments nutritifs en provenance des rivières. On observe dans toute cette région un maximum de chlorophylle profond qui est nettement dominé par les microflagellées (>55 % de la communauté). Finalement, la dernière région se situe dans la partie centrale du Détroit d'Hudson et elle est caractérisée par une forte dominance de diatomées (>80 % de l'assemblage) et par des biomasses phytoplanctoniques comparables à celles mesurées durant les floraisons automnales dans différentes zones tempérées à travers le monde. Cette dernière région subit l'influence d'une forte instabilité de la colonne d'eau due au mélange de marée.

Introduction

The first extensive report on the phytoplankton distribution in Hudson Bay and Hudson Strait came from Bursa (1961*a*), who worked on samples collected in 1953 and 1954 during the "CALANUS" expeditions in northern Hudson Bay and western Hudson Strait. He found that the phytoplankton standing crop of western Hudson Strait was considerably richer than that of the northern region of Hudson Bay. On a larger spatial scale, Anderson and Roff (1980*a*) observed a clear inshore–offshore gradient of particulate organic matter in Hudson Bay, with offshore values being two to three times lower. Significant correlations were found between phytoplankton biomass and freshwater runoff, surface temperature, salinity, depth, and distance from shore. Relatively higher chlorophyll concentrations were found around the Belcher and Sleeper islands and, in the Hudson Strait area, between Coats and Mansel islands.

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M.Harvey, J.-C. Therriault,[1] and N. Simard. Division des Sciences Océaniques, Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: TherriaultJcSci@dfo-mpo.gc.ca

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Anderson and Roff (1980*b*) also noticed the presence of a strong subsurface chlorophyll maximum characterizing the summer distribution of phytoplankton in the offshore waters of Hudson Bay. This was one of the most northerly reports of a subsurface chlorophyll maximum and one of the most highly developed. Legendre and Simard (1979) and Pett and Roff (1982) observed low nitrate levels in the deep waters of Hudson Bay, which were attributed to low or incomplete nutrient regeneration processes.

The only estimates of primary production for the surface waters of Hudson Bay are from measurements by Grainger (1982) in the southeastern part of the bay near the Belcher Islands and by Legendre and Simard (1979) in Manitounuk Sound. They estimated an annual primary production rate around 35 g $C \cdot m^{-2}$ $year^{-1}$ in both areas, which is comparable with the average value of 30 g $C \cdot m^{-2} \cdot year^{-1}$ obtained by Subba Rao and Platt (1984) for the seasonally ice-free areas of the Arctic Ocean. Legendre et al. (1982) attempted to couple the hydrodynamics with biological processes in the Manitounuk Sound. They found that the summer phytoplankton bloom dynamics in the upper region of Manitounuk Sound was under the influence of the local cycles of relative stability and instability of the water column, which are controlled by local winds and tides.

Apart from the study of Bursa (1961*a*), who examined the structure of the phytoplankton community in western Hudson Strait, the only data on the biological oceanography of Hudson Strait are those of Drinkwater and Jones (1987), who examined the relationship between the horizontal distribution of surface nutrient and chlorophyll concentrations and the occurrence of higher tidal mixing zones during a summer cruise. They observed higher chlorophyll concentrations in regions adjacent to weaker vertical density gradients corresponding to well-mixed surface waters containing higher nitrate concentrations. According to Sutcliffe et al. (1983), this nutrient enrichment of the surface waters through physical mixing in Hudson Strait probably has a measurable impact on the development of the marine food web "downstream" on the Labrador Shelf.

As suggested above, despite a long history of exploration and investigation, the biological oceanography of Hudson Bay and Hudson Strait has received very little systematic attention in the past. Most studies carried out in this northern region were of sporadic nature and geographically restricted (see reviews in Beals 1968; Dunbar 1982; Percy 1989), and the systematic relationships between water mass characteristics and biological processes have only occasionally been examined (i.e., Anderson and Roff 1980*a*; Anderson et al. 1981; Legendre et al. 1982) and never for Hudson Bay and Hudson Strait simultaneously. The present study adds further information to this general knowledge by examining the late-summer distribution of phytoplankton along a coastal transect covering a large region from Hudson Bay to Hudson Strait.

Material and methods

Study area

Hudson Bay, averaging 120 m and not exceeding 300 m in depth, is the largest inland sea (830×10^3 km^2) in the world (Prinsenberg 1984). Its drainage area is immense (3.1×10^6 km^2) and affects approximately 31% of Canada's land mass (Prinsenberg 1980). River runoff combined with seasonal ice melting contribute to a significant freshening of the surface waters in the bay, resulting in strong year-round temperature and salinity gradients. This confers a general estuarine character to Hudson Bay, with a typical two-layer circulation pattern separated by a strong pycnocline located between 5 and 25 m (Bursa 1968; Prinsenberg 1984; Roff and Legendre 1986).

This extensive intrusion of freshwater from land drainage drives a horizontal cyclonic circulation pattern in the surface layer of the bay that is characterized by fresher, less-dense waters from Foxe Basin entering between Southampton and Coats islands, mixing with the numerous local runoff plumes, and slowly circling the bay before exiting in Hudson Strait on either side of Mansel Island (Barber 1968; Anderson and Roff 1980*a*; Pett and Roff 1982; see also Fig. 1). The deep waters in the bay are arctic in type and originate from Foxe Basin and Hudson Strait (see references in Pett and Roff 1982). They also circulate in a general cyclonic pattern that is, however, much slower than in the surface layer, with a residence time between 4 and 14 years (Pett and Roff 1982) as compared with probably less than 1 year for the surface layer (estimated using Prinsenberg's 1986 current measurements). Surface temperatures in the bay reach 12°C in the summer while the deep waters always remain between 0 and 1.8°C (Roff and Legendre 1986). Salinity in the surface layer varies from 10.0 near major river systems to 30.0 in the central and northeastern part of Hudson Bay (Prinsenberg 1986) and ranges between 30.0 and 33.7 in the deep layer (Pett and Roff 1982). During winter months, the complete ice cover significantly dampens the circulation pattern (Freeman et al. 1982).

Hudson Strait is a wide and deep (~400 m) channel linking Hudson Bay and Foxe Basin with the Labrador Sea and Davis Strait. Strong tidal currents (up to 2–3 $m \cdot s^{-1}$) and high tidal elevations (amplitudes >4 m) are observed in the strait owing to resonance near the semidiurnal tidal frequency (Drinkwater and Jones 1987). According to Drinkwater (1986, 1990), these currents generate intense vertical mixing that affects the vertical stratification of the water column, the temperature and salinity properties, the surface nutrient concentrations, and the biological production processes in certain regions of the strait. The residual surface circulation pattern in Hudson Strait is dominated by three main features (Drinkwater 1986, 1990; also see Fig. 1): (*i*) an intense nearshore coastal current containing the low-salinity waters from Hudson Bay and Foxe Basin that flows southeastward along the Quebec shore and eventually exits the strait north of Cape Chidley, (*ii*) a weaker and broader current containing Labrador waters flowing northwestward on the Baffin Island side of the strait, and (*iii*) a strong southward cross-channel flow in the eastern half of the Strait. The main feature of the horizontal distribution of near-surface temperature and salinity is a marked across-channel gradient over most of the strait with higher temperatures and lower salinities to the south reflecting the influence of the outflow from Foxe Basin and Hudson Bay (Drinkwater 1986, 1990). The deep waters of the western side of the strait are almost exclusively of polar type and reflect the distinctive salinity and temperature characteristics of the water mass originating from Foxe Basin; on the eastern side of the strait, the influence of the Labrador Sea is evident (Drinkwater 1986, 1990).

Field sampling

Sampling was carried out in early September 1993 at 17 stations along a transect following the Quebec coast, from James Bay in Hudson Bay to Ungava Bay in Hudson Strait (Fig. 1). At each station, a Rosette unit equipped with a CTD (conductivity–temperature–depth) profiler (Applied Microsystems, model STD–12), an in situ fluorometer (Sea Technology), and nine 5-L GO-FLO bottles were used to obtain temperature, salinity, density, and fluorescence profiles down to about 2 m above the bottom, and water samples at nine discrete depths (0, 3, 5, 10 15, 20, 25, 30, and 50 m). The water sample collected from each depth was prefiltered through a Nitex net (333-μm mesh size) to remove larger organisms. Three 500-mL subsamples were filtered

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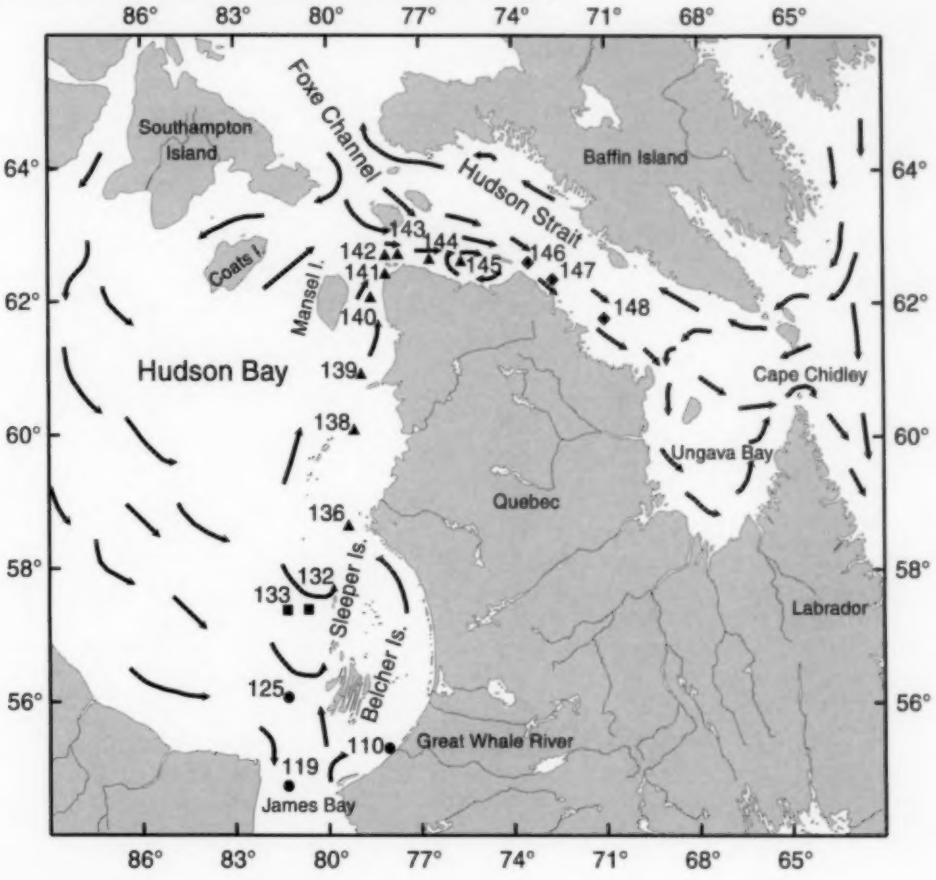
Fig. 1. Location of the sampling stations in Hudson Bay and Hudson Strait. The arrows summarize the general surface layer circulation pattern (adapted from Prinsenberg 1986 and Drinkwater 1990). The symbols indicate the positions of the four distinct groups of stations that were determined by the cluster analysis (●, group A; ■, group B; ▲, group C; ◆, group D).



through Whatman GF/F filters that were refrigerated (4°C) for later determination of chlorophyll *a* (Chl *a*) using the fluorometric method of Yentsch and Menzel (1963) as modified by Holm-Hansen et al. (1965). An additional 500-mL subsample was filtered through a precombusted Whatman GF/F filter and frozen for subsequent determination of particulate organic carbon (POC) and nitrogen (PON) contents using a Perkin Elmer CHN elemental analyzer following the method described by Sharp (1974). Some of this filtrate was also poured into three 5-mL plastic tubes and frozen in liquid nitrogen for further determination of nutrients (phosphates, nitrates, and silicates) using an Alpken autoanalyzer (Strickland and Parsons 1972). Finally, a subsample of 250 mL was preserved with 5 mL of an acidic Lugol solution, for ulterior identification and counting of the phytoplankton cells using the technique of Lund et al. (1958). Counting was done on an integrated sample from each station, which was obtained by manually combining nine aliquots of 50 mL from each depth.

Data analysis

The data were analyzed using both descriptive and multivariate analytical methods. Associations between physicochemical and biological factors were evaluated using multiple and partial correlation techniques. Ten variables were used to describe the environmental conditions at each station: surface temperature and salinity at 5 m depth; the average salinity and temperature conditions, phosphate, nitrate, and silicate concentrations in the top 50 m of the water column; an index of the vertical stratification estimated as the difference in sigma-*t* between the bottom and surface layers ($\Delta\sigma_t$); the depth of the surface mixed layer (Z_s) defined as the depth where the temperature was 1°C less than that at the surface; and the depth (Z_t) corresponding to the inferior limit of the pycnocline (Fig. 2).

Multivariate analyses were carried out on log-transformed data to identify specific phytoplankton assemblages along the sampling transect and to determine the relative contribution of the various environmental descriptors in explaining the observed structure. These statistical analyses were carried out in three steps as described in Legendre and Legendre (1984). To summarize: (*i*) a χ^2 similarity matrix was first calculated using every pair of the 80 most abundant phytoplankton species found in the overall samples; then (*ii*) the hierarchical agglomerative clustering model of Lance and Williams (1967) was used to separate groups of stations with similar species composition; and finally (*iii*) a projection in the reduced plan of the first three axes of a principal coordinate analysis was used to visualize this separation. Spearman correlations were also calculated between the principal axes and the original data to identify the phytoplankton species that contributed the most to the formation of each specific group of stations. Finally, a discriminant analysis was carried out to determine the influence of the environmental factors on the observed phytoplankton grouping.

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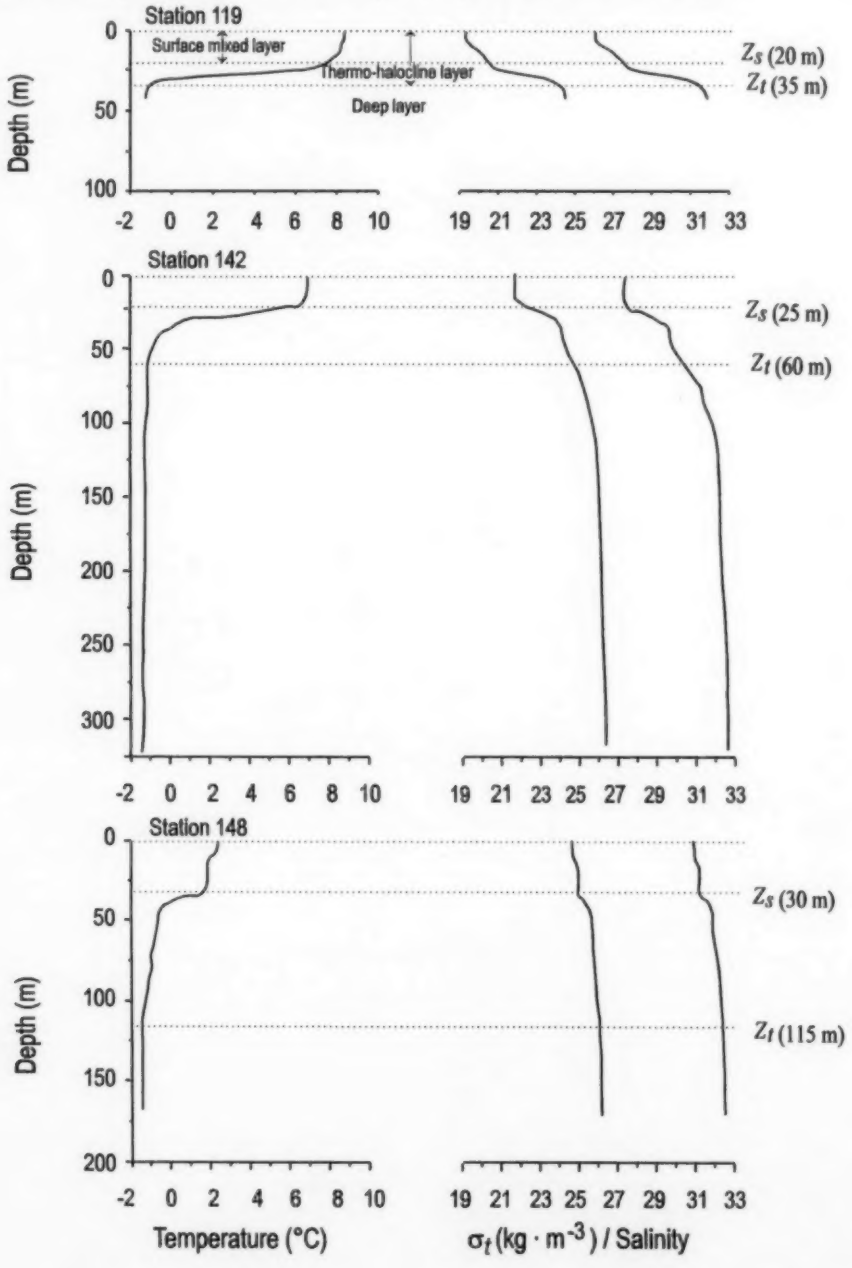
Fig. 2. Vertical profiles of temperature (T), salinity (S), and sigma-*t* (σ_t) in representative regions (stations 119, 142, and 148) of the sampling transect in early September 1993. Z_s, depth of the surface mixed layer; Z_t, depth of the thermo-halocline layer.



Results

Spatial variations of the physical and chemical variables

Strong vertical and horizontal gradients characterized the distribution of temperature, salinity, and σ_t in the upper 50 m of the water column along the sampling transect (Fig. 3). Surface salinity ranged from 25.0 at the mouth of James Bay to >31.0 at the eastern end of the sampling transect in Hudson Strait. Corresponding surface temperature varied from 8.5°C in southern Hudson Bay to 2.5°C in the central region of Hudson Strait. The σ_t variations closely followed the salinity gradient. The upper 50 m of the water column was strongly stratified in Hudson Bay and in the most westerly region of the strait, whereas it was well mixed at the eastern end of the sampling transect in Hudson Strait. The depth of the mixed layer varied from ~10 m in the southern region of the bay, to ~20 m in the

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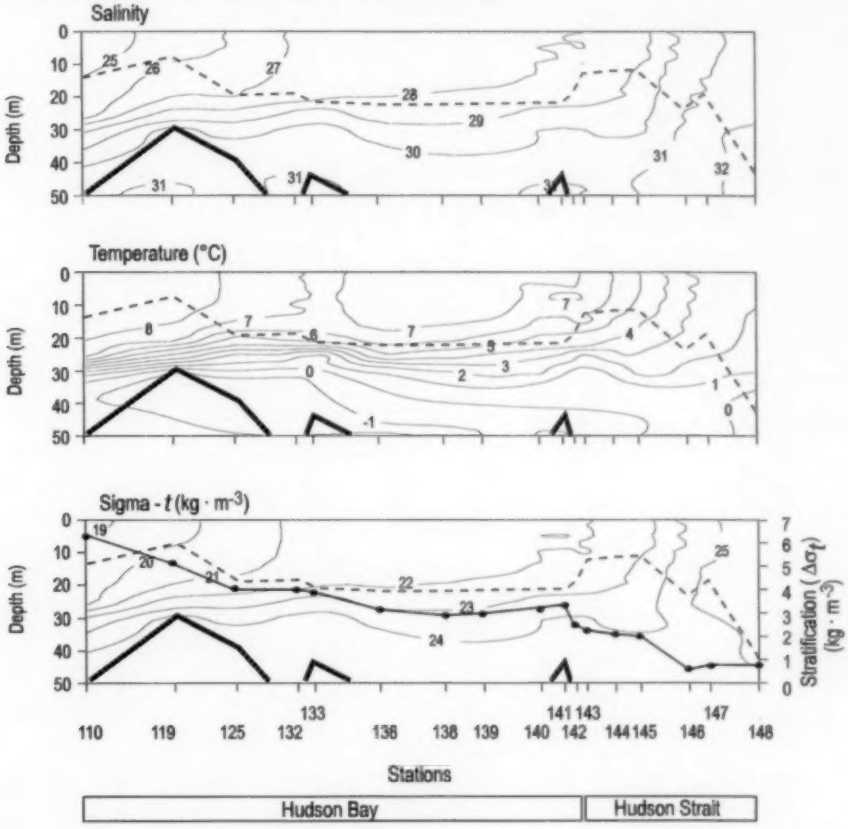
Fig. 3. Horizontal and vertical distribution of salinity, temperature, and sigma-*t* (σ_t) in the upper 50 m of the water column along the sampling transect in Hudson Bay and Hudson Strait. The depth of the surface mixed layer (- - - -), the depth of the thermo-halocline layer (——), and $\Delta\sigma_t$, the indices of stratification (—●—), are also indicated for each sampling station.


Salinity
Depth (m)
Temperature (°C)
Sigma - t (kg · m-3)
Stratification (Δσt) (kg · m-3)
Stations
110 119 125 132 133 136 138 139 140 142 141 143 144 145 146 147 148
Hudson Bay
Hudson Strait

central and northern regions of the Bay, and to ~15 m in the western part of the sampling transect in the strait; it gradually increased to more than 40 m at the most eastern sampling stations in the strait. The lower depth of the thermo-halocline gradually deepened along the transect from the mouth of James Bay towards Ungava Bay, accompanied by a similar decrease of the stratification index, $\Delta\sigma_t$ (Figs. 1 and 3). The presence of a marked transition zone between stations 142 and 146 is also apparent in Fig. 3. This frontal region separates the waters from Hudson Bay, which are typically characterized by a warmer, less saline, and well-mixed surface layer of approximately 20 m overlaying a very cold (<1.0°C) and more saline bottom layer, from the well-mixed, colder, and more saline surface layer occupying the entire upper 50 m of the water column in the central region of Hudson Strait (stations 146 to 148).

At all stations, a significant increase with depth characterized the vertical distribution of nitrates, phosphates, and silicates along the sampling transect (Fig. 4). The specific vertical profiles, however, differed depending on the local water mass characteristics along the sampling transect. The horizontal distribution patterns of the nutrients reflected well these spatial or local differences. In the surface mixed layer, very low nitrate concentrations (<0.25 μM) were observed throughout most of the bay from stations 119 to 138. Beyond station 138, surface nitrate concentrations gradually increased to values >0.5 μM in the strait. A higher surface nitrate concentration was also observed at station 110 located in the plume of the Great Whale River. Phosphate concentrations generally followed the horizontal surface distribution pattern of the nitrates, with lower values generally observed in the bay and higher values associated with the central strait region. Silicate concentrations in the surface layer, on the other hand, showed an opposite horizontal gradient, with higher values associated with the less-saline waters of the bay. A close correspondence was also generally observed between the vertical location of the nutricline and the thermo-halocline. Finally, the horizontal distribution of the nutrient profiles suggests the occurrence of mixing or upwelling zones located around station 119 at the mouth of James Bay, around stations 132–136 in Hudson Bay, and particularly in the most eastern part of our sampling transect in Hudson Strait (stations 145–148).

Spatial variations of the biological variables

In general, similar patterns of variations characterized the spatial distribution of all biological variables (Fig. 5). In the surface layer, higher values were usually observed at the most southern stations in the bay (stations 110 and 119) and, particularly, at

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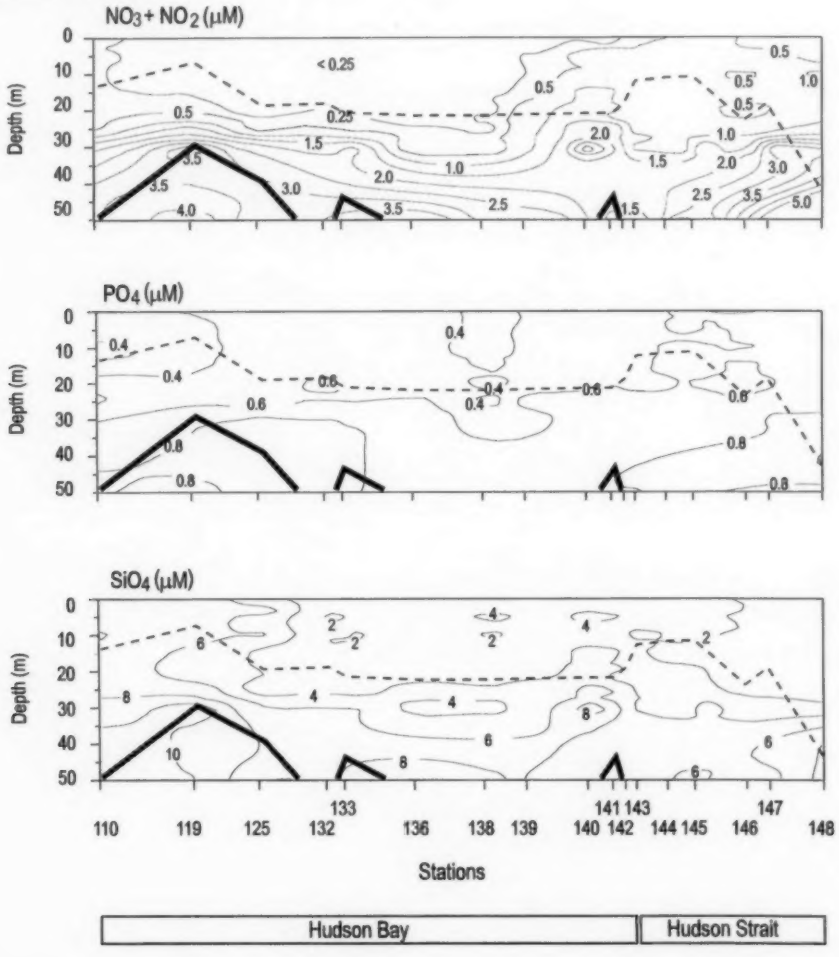
Fig. 4. Horizontal and vertical distribution of nitrates, phosphates, and silicates in the upper 50 m of the water column along the sampling transect in Hudson Bay and Hudson Strait. The depth of the surface mixed layer (- - - -) and the depth of the thermo-halocline layer (——) are also indicated for each sampling station.



the eastern end of the sampling transect in the strait (stations 145–148). The region from station 136 in the bay to station 144 in the strait was characterized by the occurrence of the lowest values of Chl *a*, fluorescence, POC, and PON in the surface mixed layer. This region was also characterized by the presence of distinct subsurface maxima in Chl *a* and fluorescence, which were located inside the thermo-halocline layer (between Z_s and Z_t). This vertical distribution pattern was, however, less evident for PON and POC. At the most eastern stations in Hudson Strait (stations 144–148), high values of Chl *a*, fluorescence, POC, and PON were found throughout the upper 50 m of the water column, suggesting important biological production associated with more intense vertical mixing in this region. A region of higher subsurface Chl *a* concentrations was also observed around stations 132 to 136 in the bay.

Diatoms were clearly dominant at the least stratified eastern end of the transect in Hudson Strait (stations 146–148), accounting for more than 80% of the phytoplankton community (Fig. 6). They also represented around 50% of the assemblage between stations 125 and 136 in the central part of the bay and between 25 and 35% of the community from station 138 to station 145, the region where the subsurface chlorophyll maximum was most apparent. Flagellates, on the other hand, clearly dominated the phytoplankton community in the most southern region of Hudson Bay (85 and 50% at stations 110 and 119, respectively), where diatoms were nearly absent (3–5%) (Fig. 6). Flagellates also accounted for more than 50% of the phytoplankton community in the central region of the bay (stations 138–145). At the most eastern end of the transect (stations 146–148) they only represented about 20% of the community. Finally, the dinoflagellates never accounted for more than about 10% of the phytoplankton community, except for station 110 in the Great Whale River plume, where they represented about 50% of the assemblage. Globally, their relative abundance decreased along the sampling transect from the bay to the strait.

The total abundance of phytoplankton cells remained more or less constant between 10×10^4 and 40×10^4 cells·L^{-1} along

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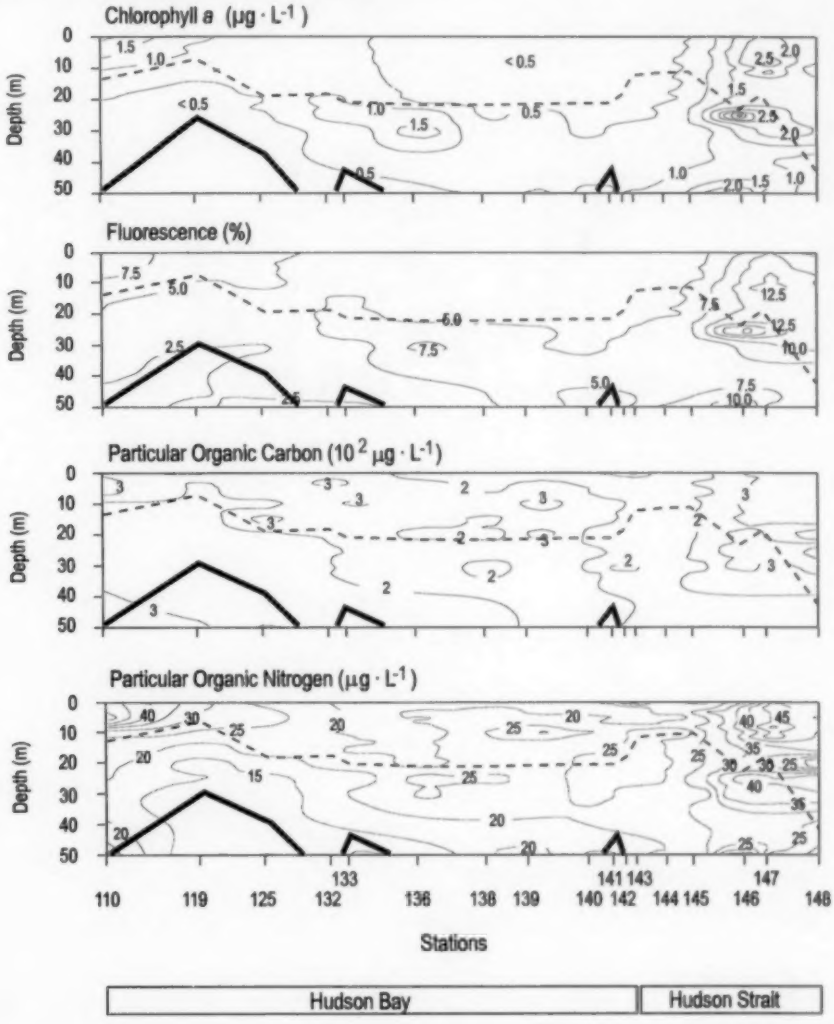
Fig. 5. Horizontal and vertical distribution of chlorophyll *a*, fluorescence, and particulate organic carbon and nitrogen in the upper 50 m of the water column along the sampling transect in Hudson Bay and Hudson Strait. The depth of the surface mixed layer (- - - -) and the depth of the thermo-halocline layer (——) are also indicated for each sampling station.


Chlorophyll a (µg · L-1)
Fluorescence (%)
Particular Organic Carbon (10 2 µg · L-1)
Particular Organic Nitrogen (µg · L-1)
Depth (m)
Stations
110 119 125 132 133 136 138 139 140 141 142 143 144 145 146 147 148
Hudson Bay
Hudson Strait

the major part of the sampling transect, that is, from station 110 to station 145 (Fig. 7).The cell numbers, however, increased sharply by almost a factor of six (averaging ca. 100×10^4 cells·L^{-1}) in the least stratified central strait region (stations 146–148). Most of this increase was accounted for by the centric diatoms. Two peaks of abundance were observed in the spatial distribution of the diatoms: a large peak on the eastern end of the sampling transect in the strait, as mentioned above, and a much smaller one at station 136. The total abundance of the dinoflagellates and of the chlorophytes, euglenophytes, prasinophytes, prymnesiophytes, and chrysophytes did not show much variation along the sampling transect. This was also generally the case for the cryptophytes, which except for a peak at station 119, showed little spatial variation in abundance. A small peak of abundance in pennate diatoms was observed at stations 146, 147, and 148, while they were practically absent from stations 110 and 119, in the southern region of Hudson Bay.

A total of 138 species were recorded in the phytoplankton of eastern Hudson Bay and Hudson Strait in early September 1993 (see the complete list of species in Simard et al. 1996). This list of species comprises 37 centric and 20 pennate diatoms, 49 dinoflagellates, and 32 flagellates (3 chlorophytes, 2 euglenophytes, 6 prasinophytes, 4 cryptophytes, 1 prymnesiophytes, 11 chrysophytes, and 5 unindentified flagellates species). Table 1, which summarizes the phytoplankton species identification, considers the 80 most abundant species that accounted for more than 97% of the total cell numbers. Table 1 indicates that 82% of the 80 most abundant phytoplankton species were present at more than 50% of the stations and 61% at more than 75% of the stations. Only two species (the chlorophyte *Diplostauron elegans* and the euglenophyte *Eutreptiella* sp.) were found at less

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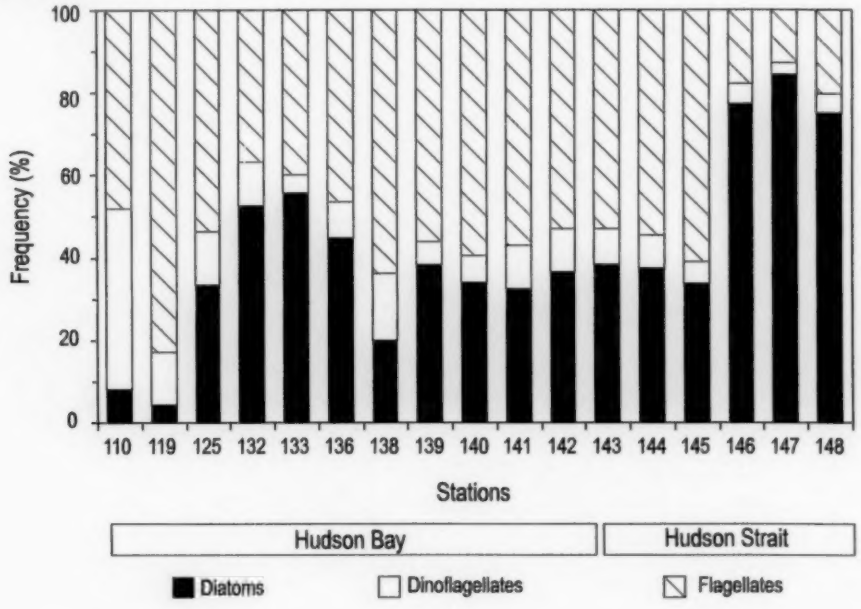
Fig. 6. Relative abundance of the principal phytoplankton groups (diatoms, dinoflagellates, and flagellates) along the sampling transect in Hudson Bay and Hudson Strait.



than 25% of the stations, whereas eight dinoflagellates, one very abundant unidentified flagellate, one prasinophyte, and two cryptophytes species were observed at all stations. The mean abundance of the various species varied between 100 and 61 300 cells·L^{-1} and 10 of the 80 most abundant species (4 centric diatoms, 1 dinoflagellates, 2 prasinophytes, 2 cryptophytes, and 1 flagellate species) had a mean abundance greater than 10 000 cells·L^{-1}.

Relationships between physical, chemical, and biological variables

There was a significant and consistent pattern in the relationships between the biological descriptors of the environment and the physical and chemical variables along the sampling transect (Table 2). For instance, except for the dinoflagellates and most of the small flagellate species, most biological descriptors were inversely correlated with silicates, temperature, and the index of stratification ($\Delta\sigma_t$) and positively correlated with most of the other physical factors. No significant correlation was found between all biological descriptors and the nitrate concentrations and, except for centric and pennate diatom numbers, the phosphate concentrations along the transect. Again, except for the flagellates and the dinoflagellates, the combination of the physical and chemical factors could usually explain a large portion of the total variance in the biological descriptors (see R^2 values in Table 2).

Spatial patterns along the sampling transect

A clustering procedure was used to objectively determine the extent of the regions and to identify the specific phytoplankton assemblage belonging to each region. The cluster analysis allowed the identification of four distinct groups (A, B, C, and D) of stations characterized by significant differences in species composition and abundance of phytoplankton along the whole sampling transect (Fig. 1). Stations of group A were located in the most southern region of Hudson Bay, in the estuary of the Great Whale River (station 110), and at the mouth of James Bay (stations 119 and 125). Stations of group B were located farther offshore, northwest of the Belcher and Sleeper islands. Group C comprised stations located in the northern part of Hudson Bay and in the western end of Hudson Strait (stations 136–145). Finally, the stations of group D occupied the most eastern end of the sampling transect in the central region of Hudson Strait (stations 146–148).

The superposition of these groups of stations on the reduced plane of the first three axes of the principal coordinate analysis (Fig. 8) showed that axis I separated group A from groups B, C, and D; axis II segregated group C from groups D and B; and axis III separated group D from group B. These three axes explained 63.5% of the total variance in species composition and abundance of phytoplankton along the sampling transect.

A Spearman correlation analysis between the first three axes of the principal coordinate analysis and the total number of cells of each species (Table 1) showed that the abundance of 20, 15, and 9 phytoplankton species were significantly correlated with axes I, II, and III, respectively. Axis I separated stations with the highest observed numbers of three dinoflagellate species (*Gonyaulax spinifera*, *Gymnodinium fusiforme*, and *Katodinium rotundatum*), one chlorophyte (*Diplostauron elegans*), one euglenophyte (*Eutreptiella* sp.), and two chrysophyte species (*Apedinella spinifera* and *Dictyocha speculum*) from the stations characterized by the highest abundance of nine diatom species (*Chaetoceros* spp., *C. atlanticus*, *C. compressus*, *C. debilis*, *C. diadema*, *C. furcellatus*, *C. septentrionalis*, *C. socialis*, and *Navicula* sp.) and one unidentified flagellate species (microflagellate species). The second axis

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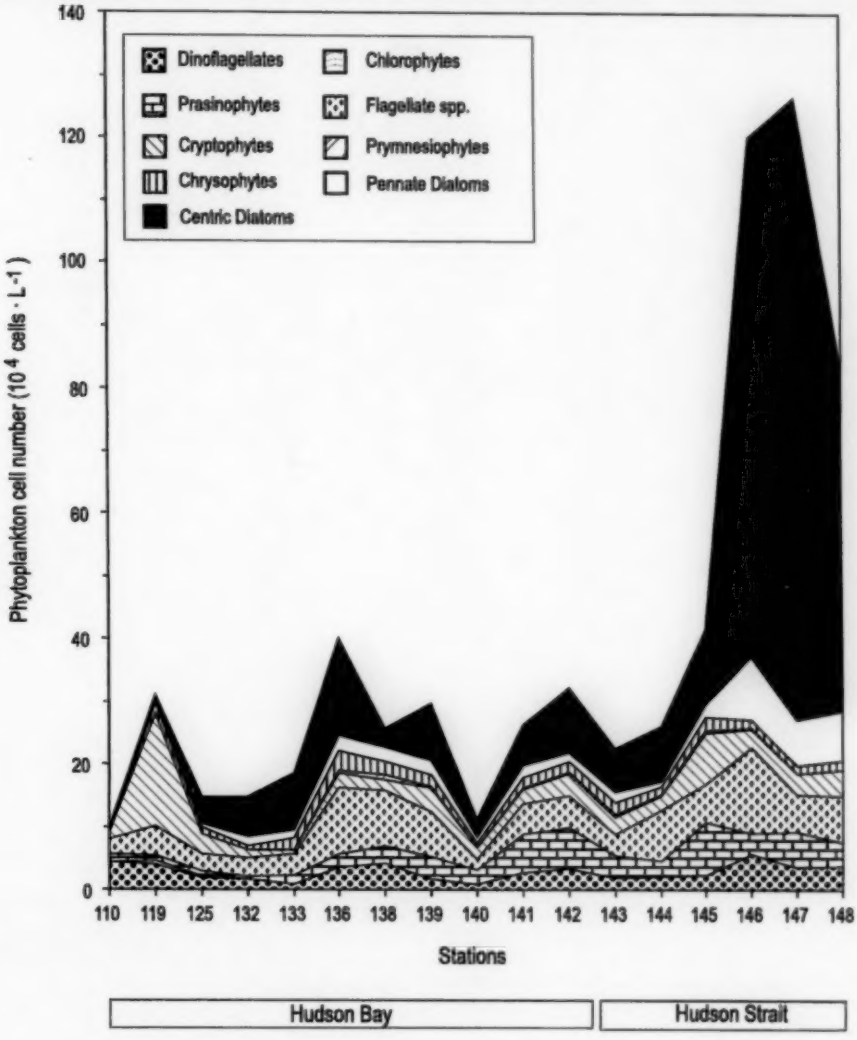
Fig. 7. Distribution of centric diatoms, pennate diatoms, dinoflagellates, chlorophytes, euglenophytes, prasinophytes, cryptophytes, prymnesiophytes, and chrysophytes along the sampling transect in Hudson Bay and Hudson Strait.



was positively associated with the total abundance of two prasinophytes (*Pachysphaera pelagica* and *Pterosperma cristatum*), one cryptophyte (*Leucocryptos marina*), and two diatoms (*Chaetoceros convolutus* f. *trisetosa* and *Thalassionema nitzchioides*) and negatively correlated with the abundance of eight diatom species (*Chaetoceros concavicornis*, *C. debilis*, *C. decipiens*, *Leptocylindrus danicus*, *Thalassiosira bioculata*, *T. nordenskioeldii*, *Cylindrotheca closterium*, and *Navicula* sp.) and two dinoflagellates (*Alexandrium ostenfeldii* and *Gymnodium* spp.). Finally, the third axis contributed to the separation of stations according to gradients in four diatoms (*Chaetoceros furcellatus*, *Cylindrotheca closterium*, *Fragilaria* sp., and *Skeletonema costatum*), two dinoflagellates (*Protoperidinium brevipes* and *Scrippsiella* sp.), one prasinophyte (*Pyramimonas* sp. 1), and two chrysophyte species (*Dinobryon balticum* and *Meringosphaera mediterranea*).

A discriminant analysis was further used to identify the environmental factors responsible for the separation of the four groups. The first two functions of this discriminant analysis explained 98% of the intergroup variance (Wilks' $\lambda = 0.0001$, $p = 0.0002$) and resulted in the proper a posteriori classification of all stations. Groups B, C, and D are discriminated along the first axis (Fig. 9) for which $\Delta\sigma_t$, Z_t, temperature at 5 m, and average temperature for the top 50 m are the main environmental variables that allowed the separation of those groups (Table 3). Group A is discriminated along the second axis, for which Z_t, $\Delta\sigma_t$, salinity at 5 m, and average silicate concentrations in the upper 50 m are the main environmental variables explaining the separation from the other groups.

An examination of the physicochemical characteristics of each assemblage (Table 4) reveals that the first assemblage of species (group A) was associated with the most highly stratified

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Table 1. Summary of phytoplankton species identification and abundance along the sampling transect in Hudson Bay and Hudson Strait.

Species	% O	Range (cells·L^{-1}, ×10^3)	Mean (SD) (cells·L^{-1}, ×10^3)	Axis I	Axis II	Axis III
Centric diatoms						
Arcocellulus cornucervis	77	0–46.6	8.0 (11.3)	–	+	–
Chaetoceros spp.	94	0–259.6	45.8 (82.0)	–0.52	–	–
Chaetoceros sp.	77	0–17.7	2.5 (4.1)	–	–	–
C. atlanticus	53	0–0.2	0.1 (0.1)	–0.70	–	–
C. concavicornis	53	0–0.4	0.1 (0.1)	–	–0.49	+
C. compressus	82	0–418.8	61.3 (119.6)	–0.64	–	–
C. convolutus trisetosa	53	0–2.1	0.5 (0.7)	–	0.65	+
C. debilis	82	0–75.5	13.2 (22.6)	0.57	–0.57	–
C. decipiens	47	0–0.3	0.1 (0.1)	–	–0.60	–
C. diadema	88	0–34.3	4.6 (8.2)	–0.71	–	–
C. furcellatus	77	0–35.4	5.1 (10.6)	–0.66	–	–0.54
C. laciniosus	35	0–1.8	0.2 (0.5)	–2.257	–	+
C. septentrionalis	94	0–13.0	2.9 (3.3)	–0.61	+	–
C. similis	77	0–2.0	0.9 (0.7)	–0.61	–	+
C. socialis	88	0–141.6	33.0 (46.4)	–0.71	+	–
C. subtilis	29	0–0.7	0.1 (0.2)	–	–	+
Leptocylindrus danicus	94	0–19.8	5.8 (6.3)	–	–0.72	+
L. minimus	71	0–52.5	4.8 (12.4)	–0.71	–	+
Skeletonema costatum	35	0–8.7	1.5 (2.6)	–	–	0.81
Thalassiosira sp.1	88	0–2.5	0.5 (0.7)	0	–	+
T. bioculata	53	0–1.4	0.2 (0.4)	0	–0.67	–
T. gravida	41	0–1.4	0.1 (0.4)	0	–	–
T. nordenskioeldii	65	0–37.8	4.6 (10.1)	–	–0.80	–
Pennate diatoms						
Cylindrotheca closterium	65	0–0.7	0.1 (0.2)	–	–0.56	–0.61
Fragilaria sp.	41	0–52.5	7.1 (15.5)	–	–	–0.58
Fragilariopsis nana	65	0–9.4	2.1 (2.4)	–	+	–
Navicula sp.	59	0–0.7	0.1 (0.2)	–0.57	–0.52	–
Nitzschia delicatissima	82	0–2.0	0.9 (0.7)	–	+	–
N. longissima	94	0–5.3	1.7 (1.4)	–	–	–
N. seriata	47	0–1.4	0.1 (0.3)	–	–	–
Pennate diatoms sp. 1	88	0–24.8	4.5 (6.3)	–0.56	–	–
Pennate diatoms sp. 2	82	0–26.5	4.6 (8.1)	–	+	–
Thalassionema nitzchioides	94	0–10.3	4.5 (3.1)	–	0.61	+
Dinoflagellates						
Alexandrium ostenfeldii	94	0–0.5	0.1 (0.1)	–1.13	–0.53	–
Amphidinium crassum	82	0–0.4	0.1 (0.1)	–	–	–
Ceratium arcticum	88	0–0.2	0.1 (0.1)	0	–	+
Cochlodinium sp.	77	0–1.1	0.1 (0.3)	–	+	–
Dinophysis acuminata	100	0–1.1	0.2 (0.3)	–	–	–
Dinoflagellate sp.	94	0–5.1	1.0 (1.3)	–	–	–
Gonyaulax spinifera	82	0–12.4	0.8 (3.0)	0.60	–	–0.813
Gymnodinium fusiforme	100	0–0.4	0.2 (0.1)	0.82	+	+
Gymnodinium spp.	100	0–0.8	0.2 (0.2)	–	–0.56	–
Gymnodium sp. 1	94	0–35.4	13.7 (9.2)	–	+	–
Gymnodium sp. 2	100	0.6–15.0	7.1 (3.5)	1.223	+	–
Katodinium glaucum	94	0–1.8	0.4 (0.5)	–	+	+
K. rotundatum	35	0–2.4	0.4 (0.8)	0.57	–	–
Dinoflagellate spp. (cysts)	65	0–0.9	0.1 (0.2)	0.57	–	–
Minuscula bipes	88	0–2.8	0.5 (0.7)	–	–	+
Oxytoxum sp.	100	0–3.5	0.8 (0.9)	–	–	–
Peridinium sp.	100	0.2–3.2	1.0 (0.8)	–	–	–
Prorocentrum minimum	53	0–1.1	0.2 (0.3)	–	–	–
Protoperidinium brevipes	100	0–0.5	0.1 (0.1)	0	+	+

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Table 1 (*concluded*).

Species	% O	Range (cells·L^{-1}, ×10^3)	Mean (SD) (cells·L^{-1}, ×10^3)	Axis I	Axis II	Axis III
P. pellucidum	71	0–0.1	0.1 (0.1)	–0.61	+	–0.49
Scrippsiella sp.	65	0–4.4	0.5 (1.2)	–0.61	–	0.72
Torodinium robustum	100	0–0.4	0.1 (0.1)	–	+	+
Chlorophytes						
Diplostauron elegans	23	0–0.6	0.1 (0.2)	0.65	–	+
Euglenophytes						
Eutreptiella sp.	18	0–5.3	0.5 (1.4)	0.65	–	+
Prasinophytes						
Pachysphaera pelagica	41	0–0.5	0.1 (0.2)	1.3	0.79	+
Prasinophytes sp.	82	0–49.8	20.0 (17.5)	–	+	–
Pseudoscourfieldia marina	77	0–3.5	1.3 (1.2)	–	–	–
Pterosperma cristatum	59	0–1.1	0.3 (0.4)	0	0.85	+
Pyramimonas sp. 1	100	2.4–33.6	10.7 (8.8)	–	+	–0.53
Flagellates						
Flagellate spp.	59	0–3.5	0.9 (1.3)	–	–	–
Flagellate sp. 1	77	0–4.1	1.6 (1.4)	–	+	–
Microflagellate spp.	100	16.6–130.5	55.1 (30.1)	–0.53	+	–
Cryptophytes						
Cryptophytes sp. 1	100	0.06–41.3	12.9 (12.1)	–	+	+
Cryptophytes sp. 2	100	2.4–127.4	21.4 (28.7)	–0.53	–	–
Cryptophytes sp. 3	47	0–1.8	0.3 (0.5)	–	–	–
Leucocryptos marina	88	0–11.2	2.7 (2.8)	0	0.62	+
Prymnesiophytes						
Chrysochromulina sp.	59	0–8.3	2.0 (2.4)	0	+	–
Chrysophytes						
Apedinella spinifera	41	0–1.2	0.2 (0.4)	0.69	+	+
Chrysophytes sp. 1	41	0–3.5	0.6 (1.0)	–	+	–
Chrysophytes sp. 2	82	0–3.5	1.4 (1.1)	–	+	+
Dictyocha speculum	88	0–0.4	0.7 (0.1)	0.51	–	+
Dinobryon balticum	88	0–14.8	4.3 (3.9)	1.2	+	0.72
D. petiolatum	65	0–1.8	0.4 (0.4)	–	+	+
Chrysophytes spp. (cysts)	82	0–8.3	2.7 (2.4)	–0.51	+	–
Meringosphaera mediterranea	82	0–2.4	1.0 (0.8)	–0.51	–	0.77
Ochromonas sp.	53	0–5.9	1.5 (1.9)	0	+	+
Pseudopedinella pyriforme	77	0–6.5	3.1 (2.3)	–	+	–

Note: The percentage of occurence (% O), range, mean abundance, and SD are presented, along with the Spearman correlation coefficients between the abundance of each species in each sample and the three axes of the principal coordinate analysis. Axes I, II, and III explained 40.3, 12.5, and 10.7% of the variance, respectively. Only significant ($p \leq 0.05$) coefficients are shown. The plus or minus signs indicate direction of nonsignificant correlations.

water column, with surface water salinity less than 26.0; groups B and C were characterized by colder and still well stratified surface waters, with salinity of ca. 28.0; and group D was characterized by the coldest and well-mixed surface waters, with salinity around 31.0.

Discussion

Phytoplankton distribution in relation to water mass characteristics

Both descriptive and multivariate analytical methods allowed the separation of the sampling stations into four distinct groups or phytoplankton assemblages that characterized well-defined areas along the various environmental gradients of the sampling transect. In other words, different parts of the sampling transect were characterized by different physical forcing functions, which through their action on surface water temperature, salinity, stratification, and mixing conditions and the resultant modifications in nutrient enrichment and in the general light conditions for photosynthesis, determined the type of phytoplankton community that could be sustained in different areas of Hudson Bay and Hudson Strait.

The first assemblage (group A) was associated with stations 110, 119, and 125 that were located in the most southern region of Hudson Bay (Fig. 1). This region is most strongly influenced by the combined freshwater runoff from James Bay and the major rivers around Hudson Bay (particularly the Great Whale River in the case of station 110). Consequently, the stations of group A were the most strongly stratified ($\Delta\sigma_t$ between 4 and 6 kg·m^{-3}) along the sampling transect, and the circulation pattern in this area of the bay was the most typically estuarine, with a 10–15 m deep surface layer comprising the warmer (8.5°C) and less saline (24.5) waters of the combined

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Table 2. Relationships between biological (chlorophyll *a*, fluorescence, POC, PON, and abundances of phytoplanktonic cells) and physicochemical descriptors in Hudson Bay and Hudson Strait in early September 1993.

Variable	NO_2+NO_3 (mM)	PO_4 (mM)	SiO_4 (mM)	Temperature (°C) 5 m	Temperature (°C) Mean of top 50 m	Salinity 5 m	Salinity Mean of top 50 m	$\Delta\sigma_t$	Z_s (m)	Z_t (m)	R^2
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	2	+	–0.57	–0.80	–0.60	0.55	0.61	–0.76	0.59	0.66	0.71
Fluorescence (%)	0	+	–0.63	–0.83	–0.61	0.58	0.62	–0.82	0.60	0.73	0.95
POC ($\mu g \cdot L^{-1}$)	0	+	–	–0.63	–	+	0.50	–0.57	0.64	+	0.35
PON ($\mu g \cdot L^{-1}$)	0	+	–0.48	–0.72	–	+	+	–0.70	0.65	0.62	0.84
Total phytoplankton abundance (cells·L^{-1})	0	+	–0.74	–0.79	–0.59	0.80	0.77	–0.88	+	0.62	0.70
Diatoms	0	0.52	–0.84	–0.86	–0.80	0.91	0.90	–0.93	+	0.69	0.83
Centric diatoms	+	0.54	–0.83	–0.86	–0.80	0.90	0.89	–0.92	+	0.69	0.81
Pennate diatoms	+	+	–0.79	–0.86	–0.72	0.89	0.88	–0.93	0.60	0.65	0.92
Flagellates	–	–	–	–	–	0.48	+	–0.48	+	+	0.23
Chlorophytes	–	–	0.55	+	0.54	–0.68	–0.66	0.50	+	–	0.81
Euglenophytes	–	–	+	+	+	–0.54	–	+	–	–	0.51
Prasinophytes	+	+	–	–	–	0.62	0.55	–0.54	+	0.49	0.00
Cryptophytes	+	+	+	–	+	+	–	–	–	+	0.12
Prymnesiophytes	–	–	+	+	+	+	+	–	–	+	0.77
Chrysophytes	–	–	–	–	–	+	+	–	–	+	0.66
Flagellate spp.	–	–	–0.60	–	–	0.58	+	–0.62	+	+	0.44
Dinoflagellates	–	–	–	–	–	+	+	–	+	+	0.49

Note: Adjusted squared multiple correlation coefficients (R^2) are also presented. Only significant ($p < 0.05$) coefficients are shown. The plus and minus signs give the direction of the nonsignificant correlations. All analyses were carried out on log-transformed data.

runoff plumes, overlaying a much colder (less than –1.0°C) and saline (~31.0) deep layer. Relatively higher phytoplankton (Chl $a > 1.0$ mg·L^{-1}) and PON concentrations were associated with this nutrient-enriched surface layer. Such an enhanced growth of phytoplankton due to freshwater has often been observed in other coastal ecosystems (e.g., Bowman and Iverson 1978; Denman and Powel 1984; Levasseur et al. 1992). As suggested by Levasseur et al. (1992) for another environment influenced by freshwater runoff, the relatively high N/C ratio (>0.12) found at stations of group A is an indication that the phytoplankton community in the surface layer of the southern region of the bay was not nutrient limited. Likewise, the strong, buoyancy-driven current associated with the estuarine circulation may have favored the accumulation of phytoplankton cells in the surface layer by reducing the effect of sedimentation processes, and the thin surface mixed layer may have also played a role to counterbalance the reduced light penetration due to the higher concentrations of total seston associated with freshwater runoff (Levasseur et al. 1992).

The phytoplankton community that was associated with the surface layer of group A stations was mainly characterized by a large dominance of dinoflagellate (i.e., *Gonyaulax spinifera*, *Gymnodinium fusiforme*, and *Katodinium rotundatum*) and small flagellate species (i.e., the chlorophyte *Diplostauron elegans*, the euglenophyte *Eutreptiella* sp., and the chrysophytes *Apedinella spinifera* and *Dictyocha speculum)* and by the relative absence of diatom species. This predominance of dinoflagellates and flagellates in regions adjacent to major river outflows was observed by Anderson et al. (1981) during their summer synoptic survey of the Hudson Bay. Runge et al. (1991) have also examined the relative importance of microflagellates as compared with diatoms at 2.5 m under the ice cover in the same area during spring 1986, and they also found that microflagellates accounted for at least 60–80% of the total number of cells during the period of 20 April – 19 June. These observations suggest that a specific brackish–marine phytoplankton community is always associated with the combined freshwater runoffs from James Bay and from the major rivers around Hudson Bay. However, it is of interest to note that, except for *Dinobryon balticum*, which was found at all stations of Group A, no typically freshwater species were found in this area of the bay. This observation diverges somewhat from Gerrath et al. (1980), who observed higher cell counts of typically freshwater species that were associated with regions of greater freshwater influence in the southwestern region of the bay.

The second assemblage (group B) comprises stations 132 and 133, which are also located offshore in the southern region of Hudson Bay, northwest of the Belcher and Sleeper islands. Strong stratification conditions were still present in this region ($\Delta\sigma_t \approx 3.8$ kg·m^{-3}), but the influence of the freshwater runoff had already been attenuated by a significant deepening of the surface mixed layer (~20 m) and of the lower depth of the thermo-halocline. Consequently, relatively lower temperature (~6.0°C) and salinity (~28.0) conditions were encountered in the surface mixed layer. Chlorophyll *a* values in the surface layer of group B stations were also relatively lower (~0.5 $\mu g \cdot L^{-1}$), but a significant subsurface maximum with chlorophyll values >1.0 $\mu g \cdot L^{-1}$ could be observed in the thermo-halocline layer.

The phytoplankton assemblage in the upper 50 m at group B stations was characterized by the dominance of diatoms and flagellates, which represented 54 and 38% of the total cell numbers, respectively. The occurrence of dinoflagellates was marginal, especially at station 133. The species that contributed the most to the segregation of this group were the diatoms *Chaetoceros concavicornis*, *C. debilis*, *C. decipiens*, *Leptocylindrus danicus*, *Thalassiosira bioculata*, *T. nordenskioeldii*, and *Navicula* sp. and the dinoflagellates *Alexandrium ostenfeldii*

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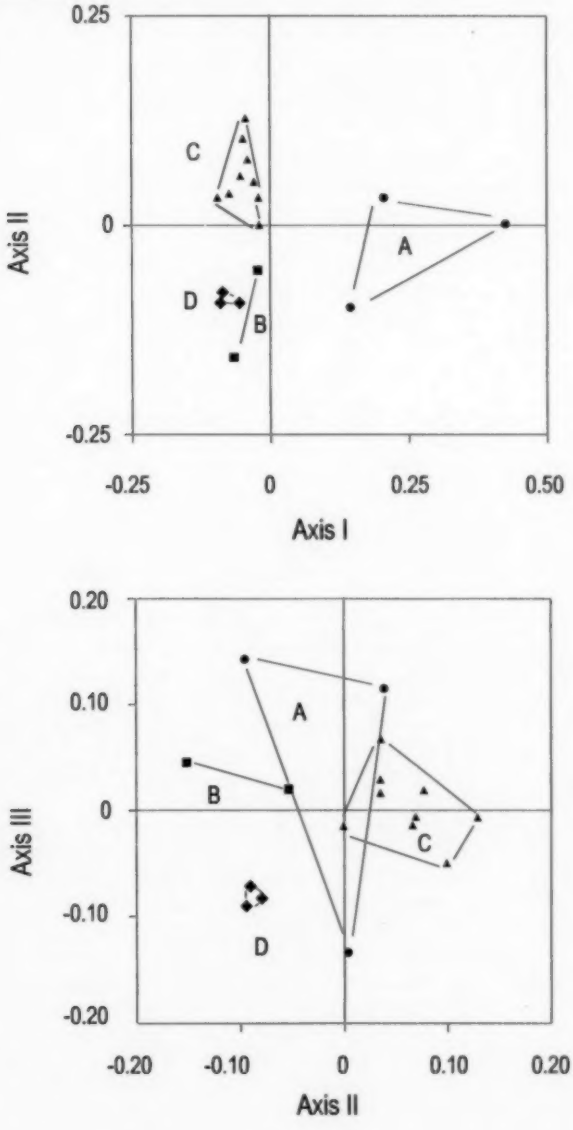
Fig. 8. Results of the principal coordinate analysis on the phytoplankton of Hudson Bay and Hudson Strait. The simultaneous projection of the stations on the first three axes of the principal coordinate analysis are presented in this figure. Clustering of stations was obtained using a hierarchical agglomerative clustering model (average linkage method). Groups were formed at the similarity level of 1.0.



and *Gymnodium* sp. 1. Furthermore, the highest diatom abundances (88 000 cells·L^{-1}) along the sampling transect in the bay were recorded at these stations. These abundances compare well with the maximum values of 100 000 cells·L^{-1} found by Anderson et al. (1980*a*) in the same region. The higher phytoplankton biomass observed in the region northwest of the Belcher and Sleeper islands probably resulted from the enhanced mixing and upwelling conditions that are encountered

Fig. 9. Results of the discriminant analysis on the phytoplankton of Hudson Bay and Hudson Strait. The position of the four groups of stations and of their centroids (*) with respect to the first two discriminant axes are shown.

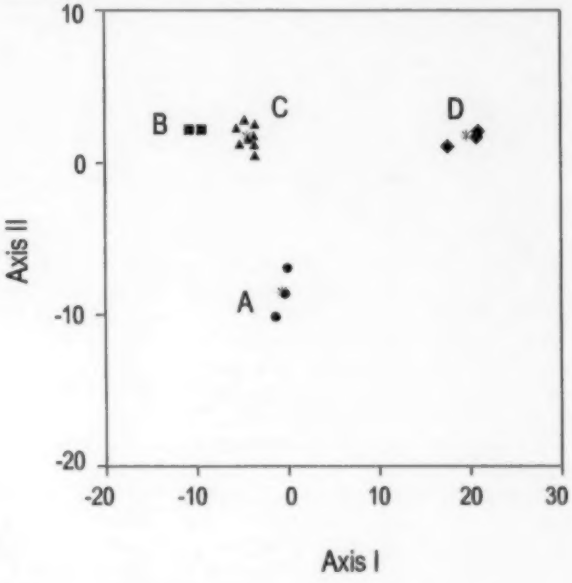


Table 3. Contribution of environmental variables (standardized canonical coefficients) to the formation of the first discriminant axes separating the groups of stations.

Variables	Axis I	Axis II
NO_3+NO_2	1.127 (8)	0.069 (9)
PO_4	0.761 (10)	–0.693 (8)
SiO_4	1.032 (9)	–1.456 (4)
Salinity (5 m)	–1.602 (6)	1.998 (3)
Salinity (mean, top 50 m)	–1.231 (7)	0.160 (10)
Temperature (5 m)	–2.152 (4)	1.041 (5)
Temperature (mean, top 50 m)	2.156 (3)	–1.030 (6)
Z_s	2.030 (5)	0.703 (7)
Z_t	–3.123 (2)	3.010 (1)
$\Delta\sigma_t$	–4.974 (1)	2.420 (2)

Note: Values in parentheses indicate rank in decreasing order of importance. Axes I and II explained 84.0 and 14.3% of the variance, respectively.

in this region owing to the interaction between the general cyclonic circulation and the "island mass effect" (Doty and Oguri 1956).

The third phytoplankton assemblage (group C) occupied the largest region along the sampling transect, from station 136 in the middle of Hudson Bay to station 145 in the western region of Hudson Strait. The water column in this large region was still strongly stratified, with a mixed layer depth located around 20 m ($\Delta\sigma_t \approx 3.0$ kg·m^{-3}) in the bay and around 15 m ($\Delta\sigma_t \approx 2.0$ kg·m^{-3}) in the western region of the strait. Mixed layer temperature and salinity conditions characterizing group C stations were relatively constant, around 6–7°C and 27.0–28.0, respectively. Our results also present evidence that the western strait stations were located in a transition zone

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Table 4. Mean, with SD given in parentheses, of environmental variables that better discriminated groups of phytoplanktonic species.

Variables	Assemblages of phytoplanktonic species			
	A ($n = 3$)	B ($n = 2$)	C ($n = 9$)	D ($n = 3$)
$\Delta\sigma_t$	5.29 (1.11)	4.04 (0.08)	2.89 (0.47)	0.89 (0.14)
Z_t(m)	43.33 (5.77)	57.50 (10.61)	86.11 (33.52)	141.67 (38.19)
Temperature, 5 m (°C)	8.31 (0.39)	6.75 (0.63)	6.54 (1.12)	2.60 (0.33)
Temperature, mean top 50 m (°C)	4.05 (0.90)	2.46 (0.40)	3.24 (0.51)	1.70 (0.50)
Salinity, 5 m	25.21 (1.07)	27.43 (0.50)	28.14 (0.34)	31.00 (0.32)
SiO_4 (μM)	6.93 (1.65)	4.29 (0.49)	3.95 (1.54)	1.89 (0.58)

separating the warmer, less-saline, and well-stratified waters leaving the bay from the colder, saltier, and well-mixed waters originating from the western side of the strait. Very low chlorophyll values were observed in the surface mixed layer throughout the group C stations. However, the occurrence of a well-developed subsurface chlorophyll maximum characterized this region of the sampling transect. These observations are in agreement with the study of Anderson and Roff (1980*b*), which reported a widespread chlorophyll maximum below the pycnocline in most of the offshore region of Hudson Bay. Although not reported frequently for northern waters, the occurrence of a subsurface chlorophyll maximum in stratified waters is a common phenomenon (see review in Vandevelde et al. 1987). One of the various mechanisms involved in explaining this phenomenon is the decreased sinking rates of phytoplankton cells at the pycnocline level, where they can find higher nutrients levels at light conditions that can still sustain photosynthesis.

The phytoplankton assemblage in the upper 50 m of the water column in this large region of the sampling transect was dominated by flagellate and diatom species that composed 57 and 36% of the total cell numbers, respectively. Dinoflagellates only accounted for 7% of the phytoplankton community. The species that contributed the most to the segregation of this group are the prasinophytes *Pachysphaera pelagica* and *Pterosperma cristatum*, the cryptophyte *Leucocryptos marina*, and the diatoms *Chaetoceros convolutus* f. *trisetosa* and *Thalassionema nitzchioides*. On average, the diatom and dinoflagellate cell counts obtained along the east coast of the Hudson Bay were similar to those of Anderson et al. (1981) for the same region. They were, however, much higher than those obtained by Bursa (1961*a*) and Anderson et al. (1981) for the northern Hudson Bay and western Hudson Strait regions. The low phytoplankton biomass observed from station 136 in the middle of Hudson Bay to station 145 in the western region of Hudson Strait probably result from (*i*) the persistent vertical stratification of Hudson Bay, (*ii*) the low nutrient supply from freshwater runoff, and (*iii*) the low in situ nutrient regeneration within the mixed layer during the summer (Anderson and Roff 1980*a*, Anderson et al. 1981).

The phytoplankton assemblages from stations 146, 147, and 148 form group D, located at the most eastern part of the sampling transect in Hudson Strait. This region showed biological and physicochemical characteristics that were drastically different from those of the other three groups. The upper 50 m of the water column in the central strait region was well mixed ($\Delta\sigma_t \approx 0.8$ kg·m^{-3}) up to about 40 m, with the coldest (~2.5°C) and saltiest (~31.5) waters encountered at the surface along the sampling transect. Much higher chlorophyll and nutrient concentrations were also observed throughout the upper 50 m of the water column at these stations. These conditions are indicative of an enhanced biological production triggered by more active hydrodynamic processes occurring in the eastern region of the sampling transect in Hudson Strait. Drinkwater and Jones (1987) examined the relationship between regions of high tidal mixing and the horizontal distributions of surface chlorophyll and nutrients during a summer cruise in the strait and found higher Chl *a* concentrations (>1 μg·L^{-1}) associated with regions of weak vertical density gradients and high nitrate concentrations. Peak Chl *a* concentrations around 4 μg·L^{-1} were found adjacent to well-mixed waters, where higher surface nitrate concentrations (>4 μM) could be found. These conditions are similar to what we observed for group D stations.

As expected, the largest abundances of phytoplankton were also observed in the central strait region. The phytoplankton assemblage of group D was largely dominated by small diatom species, which composed more than 80% of the phytoplankton community. The total cell counts in this group also dramatically outnumbered those of the other groups, with average values around 8.8×10^5 cells·L^{-1} in the central strait region as compared with average values around 1.2×10^5 cells·L^{-1} in the western side of Hudson Strait and in Hudson Bay. These diatom cell numbers are also several orders of magnitude higher than those found for the whole Hudson Bay by Anderson et al. (1981) and in northern Hudson Bay and western Hudson Strait by Bursa (1961*a*). The diatom numbers of group D were comparable with those observed during an autumn bloom situation in many temperate areas (see reviews in Levasseur et al. 1992 and Therriault and Levasseur 1985).

Apart from *Chaetoceros furcellatus*, which is the dominant diatom species at group D stations, the diatom species that contributed to the separation of this group were the same as for group B (*Chaetoceros concavicornis*, *C. debilis*, *C. decipiens*, *Leptocylindrus danicus*, *Thalassiosira bioculata*, *T. nordenskoeldii*, and *Navicula* sp.), where better growth conditions for diatoms have also been observed owing to enhanced mixing conditions associated to the island effect. This large dominance of small diatoms observed in more active hydrodynamic regions cannot be compared with any other study, since no similar work has ever been done. However, the dominance of small diatoms in well-mixed environmental conditions is well established (e.g., Pingree et al. 1978; Demers et al. 1986; Levasseur et al. 1992). Enhanced vertical tidal mixing (Drinkwater and Jones 1987) contributing to a continuous upward flow of nutrients is probably the factor explaining the high phytoplankton productivity observed within this area.

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Species composition

Seven diatom species (*Arcocellulus cornucervis*, *Chaetoceros similis*, *C. subtilis*, *Fragilariopsis nana*, *Haslea crucigeroides*, *H. kjellmannii*, and *Fragilariopsis cylindrus*) are reported here for the first time in the open waters of Hudson Bay and Hudson Strait. The addition of these species to the 182 already reported by Roff and Legendre (1986) brings the total number of diatom species to 189 for the open waters of Hudson Bay and Hudson Strait. Nine other dinoflagellate species (*Alexandrium ostenfeldii*, *Amylax triacantha*, *Cladopyxis claytonii*, *Dicroerisma psilonereiella*, *Gymnodinium fusiforme*, *Katodinium rotundatum*, *Minuscula bipes*, *Phalacroma ruudi*, and *Prorocentrum minimum*) can also be added to the list of 119 species previously reported for Hudson Bay and Hudson Strait by Bursa (1961*a*), Legendre and Simard (1979), Anderson et al. (1981), and Roff and Legendre (1986). Finally, nine flagellate species (the prasinophytes *Pachysphaera pelagica*, *Pseudoscourfieldia marina*, *Pterosperma cristatum*, and *Pyramimonas* sp. 1; the chryptophyte *Leucocryptos marina;* the prymnesiophyte *Chrysochromulina* sp.; and the crysophytes *Apedinella spinifera*, *Meringosphaera mediterranea*, and *Pseudopedinella pyriforme*) are also new additions to the previous list of 50 species and 10 genera reported by Roff and Legendre (1986).

Therefore, considering the 371 phytoplankton species previously reported by Roff and Legendre (1986) and the 25 new species added by this study, the taxonomic list of phytoplankton collected from the open waters of the Hudson Bay – Hudson Strait system is so far composed of 396 taxa. The addition of the 88 species from the winter ice flora of Manitounuk Sound (Poulin and Cardinal 1982*a*, 1982*b*, 1983) brings the total number of phytoplankton species to 484 taxa. This relatively high diversity in the phytoplankton community in the Hudson Bay – Hudson Strait system, despite its evident arctic character, rivals reports of phytoplankton diversity anywhere in the world's oceans (Roff and Legendre 1986). This high diversity probably reflects the complex circulation processes characterizing the hydrodynamics of this northern region, particularly the area where the bay meets the Arctic and North Atlantic waters via Hudson Strait and Foxe Channel. This observation is also supported by the fact that marine diatoms and dinoflagellates found in Hudson Bay do not differ significantly in species composition from those reported in Arctic and North Atlantic waters by Bursa (1961*b*), Hulburt and MacKenzie (1971), and Hsiao et al. (1977) and are a mixture of arctic, boreal, and temperate phytoplankton species (Anderson et al. 1981).

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DISCUSSION

Comment — Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (^{137}Cs)[1]

Jixiang He and Donald J. Stewart

Rowan and Rasmussen (1996) reported that mature fish have two- to four-fold higher activity than immature fish, so the former do not follow the rule that specific respiration rate decreases with increasing body mass. Their modeling results contradict Koch and Wieser's (1983) experimental results in which gonadal synthesis drastically reduced swimming activity. We find that Rowan and Rasmussen's results may come from an error in their model development. To make the point clear, we repeat Rowan and Rasmussen's theoretical analyses (using their equation numbers) as follows:

$$(2) \quad W_t = W_0 e^{Gt}$$

where W is body mass (g), t is time, and G is the specific growth rate ($g \cdot g^{-1} \cdot t^{-1}$). Notice that $G = (\log_e(W_t) - \log_e(W_0))/t$, and it does not include reproduction.

$$(3) \quad dQ/dt = \alpha[^{137}Cs_f]CW_t - (E + D)Q$$

where Q is ^{137}Cs burden (Bq), D is the specific radioactive decay of ^{137}Cs ($Bq \cdot Bq^{-1} \cdot t^{-1}$), E is the elimination rate of ^{137}Cs ($Bq \cdot Bq^{-1} \cdot t^{-1}$), C is the specific rate of food consumption ($g \cdot g^{-1} \cdot t^{-1}$), $[^{137}Cs_f]$ is Cs concentration in food ($Bq \cdot g^{-1}$, in Rowan and Rasmussen's paper, they give $Bq \cdot kg^{-1}$), and α is the assimilation efficiency of ^{137}Cs. Combining eqs. 2 and 3 gives

$$(4) \quad dQ/dt = \alpha[^{137}Cs_f]CW_0e^{Gt} - (E + D)Q$$

Integrating eq. 4 gives

$$(5) \quad Q_t = \frac{\alpha[^{137}Cs_f]CW_0(e^{Gt} - e^{-(E+D)t})}{(G + E + D)} + Q_0e^{-(E+D)t}$$

In Rowan and Rasmussen's paper, the last term is incorrectly given in the numerator of the first term for both eqs. 5 and 6. We suspect that was a typographical error because their eq. 7 was mathematically correct but cannot be derived from their eqs. 5 and 6. However, that is not the main problem that we want to discuss here. For mature fish, Rowan and Rasmussen (1996) consider that "if spawning occurs during the period modelled, the ^{137}Cs lost in eggs and sperm (Q_g) should be subtracted." Therefore, they have

$$(6) \quad Q_t = \frac{\alpha[^{137}Cs_f]CW_0(e^{Gt} - e^{-(E+D)t})}{(G + E + D)} + Q_0e^{-(E+D)t} - Q_g$$

Finally, they solve for the specific rate of consumption as

$$(7) \quad C = \frac{(Q_t - Q_0e^{-(E+D)t} + Q_g)(G + E + D)}{\alpha[^{137}Cs_f]W_0(e^{Gt} - e^{-(E+D)t})}$$

The biological problem is the inconsistency between eqs. 2 and 6. The specific rate of growth (G) is calculated from the difference between somatic masses W_t and W_0. Thus, the ^{137}Cs consumption, assimilation, decay, and elimination in eqs. 2, 3, 4, and 5 do not account for any ^{137}Cs in eggs and sperm. For immature fish, the treatment in eqs. 6 and 7 does not have any effect on calculation of consumption because Q_g is zero. For mature fish, the specific rate of consumption (C) in eqs. 3, 4, and 5 is different from the "specific rate of consumption" in eqs. 6 and 7. If reproductive losses (Q_g) are to be subtracted during the calculation of consumption (eqs. 6 and 7), then reproduction should be included in eqs. 2–4. This inconsistent treatment of reproduction may fully explain discrepancies between their model results for mature fishes and those from more traditional energetics-modeling approaches.

According to eqs. 2–7, the specific rate of consumption might have been underestimated because the time scale in their study was 1 year. In eq. 5, $\alpha[^{137}Cs_f]CW_0e^{Gt}$ is the ^{137}Cs assimilation at the end of a year, while $\alpha[^{137}Cs_f]CW_0e^{-(E+D)t}$ is ^{137}Cs elimination and decay based on ^{137}Cs assimilation at the beginning of the year. It is clear that elimination and decay are underestimated when the time scale is a year rather than a shorter interval. Thus, eq. 7 might underestimate the specific consumption rate. Rowan and Rasmussen (1996) claimed that their method could be used on a weekly time scale, so the above problem would be solved. We agree that an exponential growth function can and should be used only for intervals much shorter than a year (Ricker 1975). We wonder whether or not individual variations in ^{137}Cs burden will confound the patterns suggested by eqs. 2–7 when short time scales are used.

The ^{137}Cs elimination rate (E; $Bq \cdot Bq^{-1} \cdot t^{-1}$) is an interesting term. According to Rowan and Rasmussen (1996), it is a negative

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J. He and D.J. Stewart. State University of New York, College of Environmental Science and Forestry, 302 Illick Hall, 1 Forestry Drive, Syracuse, NY 13210, U.S.A.

[1] Paper by D.J. Rowan and J.B. Rasmussen. 1996. Can. J. Fish Aquat. Sci. **53**: 734–745.

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function of body mass and a positive function of temperature; therefore, it is similar functionally to respiration but different from excretion and egestion in fish bioenergetic models (Kitchell et al. 1977; Stewart et al. 1983). Johannes (1964) indicated that the rate of excretion of dissolved phosphorus per unit body mass decreases as body mass increases for marine animals. Edwards (1967) indicated that elimination of ^{65}Zn is a function of specific rate of respiration in plaice (*Pleuronectes platessa* L.). Can we reject the possibility that ^{137}Cs elimination is related to respiration? The primary objective of Rowan and Rasmussen's study was to develop a better method to estimate respiration related to activity. They use eqs. 2–7 to estimate consumption and then use fish bioenergetic models to calculate respiration. If we cannot reject the possibility that ^{137}Cs elimination is a function of respiration, then Rowan and Rasmussen's method will suffer from circularity.

Balancing a fish energy budget is sufficiently complex that no single approach can be totally free of bias. We apply fish bioenergetic models as comprehensive tools to improve our understanding of complex living systems and how they should be managed. We believe that application of multiple approaches to that larger goal can help to test alternative hypotheses and, ultimately, to minimize assumptions. Rowan and Rasmussen's (1996) study is important in that it introduces a new technique for evaluating in situ energetics of fishes. As applied, however, it suffers from the overly simplified exponential growth model and inconsistent treatment of reproduction. We look forward to further tests and applications of this new tool because, if it works, we all stand to benefit.

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Reply — Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (^{137}Cs)[1]

David J. Rowan and Joseph B. Rasmussen

In a recent paper (Rowan and Rasmussen 1996), we introduced a new method for estimating metabolic costs related to activity in wild fish. The main finding of that study is that the activity of fish, especially mature fish, is much higher than commonly assumed in most bioenergetic models. He and Stewart (1997) suggest that these conclusions are the result of several problems with model formulation that may lead to overestimation of activity. We will show that these objections are due to misconceptions concerning the model development and flawed mathematical analysis of our models. We also show that the contribution of the subject parameters to the estimation of activity is weak and that even extremely conservative model assumptions do not substantially alter our original findings.

Specifically, He and Stewart (1997) raise several questions regarding model formulation and we list them here: (*i*) there is a typographical error in eqs. 5 and 6; (*ii*) eq. 1 does not include gonadal growth; (*iii*) gonadal ^{137}Cs is excluded from the mass balance; (*iv*) the elimination and radioactive decay of ^{137}Cs is underestimated; and (*v*) the elimination of ^{137}Cs may be related to metabolic rate, introducing circularity.

As noted by He and Stewart (1997), there is a typographical error in eqs. 5 and 6 (Rowan and Rasmussen 1996). Equations 5 and 6 should have read:

$$(5)\quad Q_t = \frac{\alpha[^{137}Cs_{food}]\, Cw_0\,(e^{Gt} - e^{-(E+D)t})}{(G+E+D)} + Q_0 e^{-(E+D)t}$$

and

$$(6)\quad Q_t = \frac{\alpha[^{137}Cs_{food}]\, Cw_0\,(e^{Gt} - e^{-(E+D)t})}{(G+E+D)} + Q_0 e^{-(E+D)t} - Q_{gonad}$$

where Q_0 and Q_t are the initial and final burdens of ^{137}Cs in whole fish (Bq), α is the assimilation efficiency of ^{137}Cs from food (fraction), $[^{137}Cs_{food}]$ is the concentration of ^{137}Cs in food (Bq·kg^{-1}), C is the consumption rate (g food·g body weight^{-1}·day^{-1}), w_0 is the initial fish weight (kg), G is the specific growth rate (g·g^{-1}·day^{-1}), E is the elimination rate of ^{137}Cs (Bq·Bq^{-1}·day^{-1}), D is the radioactive decay rate of ^{137}Cs (Bq·Bq^{-1}·day^{-1}), t is time (days), and Q_{gonad} is the gonadal burden of ^{137}Cs lost during spawning (Bq). Equation 7, however, is correct as presented (Rowan and Rasmussen 1996).

The main objection of He and Stewart (1997) concerns the addition of the term Q_{gonad} in eq. 5, rather than in the earlier differential equation, eq. 3 (Rowan and Rasmussen 1996). This has lead to several misconceptions regarding the treatment of somatic and gonadal growth and the loss of gonadal ^{137}Cs. First, growth is introduced into the mass balance of ^{137}Cs to express consumption as a specific rate (g food·g body weight^{-1}·day^{-1}). We did not explicitly include gonadal growth because the extent to which allometric relationships (e.g., resting metabolism, ^{137}Cs elimination) would be affected by gonad weight is unclear. We did enter gonadal growth where it directly counts, that is in the energy mass balance (Rowan and Rasmussen 1996, eq. 1). As for our introduction of the gonadal ^{137}Cs at the end of our derivation, we collected most of our fish in late spring after spawning and, for our model, assumed that the burden of ^{137}Cs in gonadal tissue was lost immediately before collection. This means that somatic and gonadal ^{137}Cs was treated as one pool, subject to decay and elimination throughout the modeled interval. Thus, the assertion of He and Stewart (1997) that gonadal ^{137}Cs is excluded from the mass balance is unfounded.

An alternative model formulation is possible, where a term for the loss of ^{137}Cs to gonads is entered in the differential equations. This alternative model assumes that a constant proportion of ^{137}Cs is lost each day to gonads (K; Bq·Bq^{-1}·day^{-1}) with no exchange back to the fish:

$$(8)\quad \frac{dQ_{fish}}{dt} = \alpha\,[^{137}Cs_{food}]\, Cw - (E+D+K)Q_{fish}$$

and

$$(9)\quad \frac{dQ_{gonad}}{dt} = K^*Q_{fish} - D^*Q_{gonad}$$

Integrating eq. 8 yields:

$$(10)\quad Q_t = \frac{\alpha[^{137}Cs_{food}]\, Cw_0\,(e^{Gt} - e^{-(E+D+K)t})}{(G+E+D+K)} + Q_0 e^{-(E+D+K)t}$$

and rearranging:

$$(11)\quad Q_t = \frac{\alpha[^{137}Cs_{food}]\, Cw_0 e^{Gt}\,(1 - e^{-(G+E+D+K)t})}{(G+E+D+K)} + Q_0 e^{-(E+D+K)t}$$

This is perhaps a better way to model ^{137}Cs because there is no assumption about the timing of the release of gonads.

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D.J. Rowan. Department of Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523-1673, U.S.A.
J.B. Rasmussen. Department of Biology, McGill University, Montréal, QC H3A 1B1, Canada.

[1] Reply to comment by J. He and D.J. Stewart. 1997. Can. J. Fish. Aquat. Sci. **54**: 1953–1954.

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We recalculated some of our activity estimates for mature fish using eq. 11 and compared these with the estimates from eq. 7 (Rowan and Rasmussen 1996). We found little difference between activity estimates from eq. 11 and the original estimates. Estimates made using eq. 11 tended to be 95–99% of the original estimates, with the exception of female lake trout (*Salvelinus namaycush*) from Great Slave Lake, which were only 80% of the original estimate. However, this new activity estimate of 3.7 is still over twice that from swimming speed studies. Thus, the original treatment of gonadal ^{137}Cs is valid and supported by alternative approaches. This was, of course, to be expected in that activity in our model is least sensitive to the parameters gonadal somatic index and the concentration of ^{137}Cs in gonadal tissue (Rowan and Rasmussen 1996, Table 7). In fact, when loss of ^{137}Cs to gonads is ignored, activity estimates, are still over 90% of those estimates including loss of ^{137}Cs to gonads.

He and Stewart (1977) also suggest that the annual energy budgets that we calculated oversimplified growth. We agree that this is a simple representation of a complex system and that seasonal data on ^{137}Cs in fish and their food, as well as growth, would have been preferable. However, such refinements are unlikely to alter the findings we reported earlier (Rowan and Rasmussen 1996). In fact, we are currently analyzing a seasonal data set from biweekly samples, where estimates of consumption and activity are made on daily time steps. These estimates agree well with those reported earlier.

As the comment of He and Stewart (1997) is directed at a perceived overestimation of activity by our method, the next two objections are most curious, for if He and Stewart (1997) were right, we would have underestimated activity. In the first of these concerns, He and Stewart (1997) state that "elimination and decay are underestimated," but this is based on their flawed analysis of the intake term in eq. 5:

$$(12)\quad \frac{\alpha[^{137}Cs_{food}]\, Cw_0\, (e^{-Gt} - e^{-(E+D)t})}{(G+E+D)}$$

He and Stewart (1997) presumably reached this erroneous conclusion when, ignoring the denominator, they split the intake term (eq. 12) into $\alpha[^{137}Cs_{food}]\ Cw_0e^{Gt}$ and $\alpha[^{137}Cs_{food}]\ Cw_0e^{-(E+D)t}$, which they term "$^{137}Cs$ assimilation" and "^{137}Cs elimination and decay," respectively. Because body size does not increase with time in the latter term, He and Stewart (1997) concluded that our model underestimated loss. However, if the intake term had been properly rearranged, He and Stewart (1997) would not have made their error:

$$(13)\quad \frac{\alpha[^{137}Cs_{food}]\, Cw_0e^{Gt}}{(G+E+D)}\,(1 - e^{-(G+E+D)t})$$

The terms in eq. 13 are more properly referred to as $\alpha[^{137}Cs_{food}]\ Cw_0e^{Gt}$, ^{137}Cs uptake; $(G+E+D)$, ^{137}Cs loss; and $(1 - e^{-(G+E+D)t})$, degree of equilibrium. Thus, there is no underestimation of elimination and radioactive decay and no underestimation of consumption. Although, if we had underestimated consumption, we would have underestimated activity rather than overestimated activity as claimed by He and Stewart (1997).

The last and most interesting objection raised by He and Stewart (1997) concerns the elimination rate of ^{137}Cs and whether it may be related to total metabolic rate, thus making our analysis circular. The most compelling evidence that the elimination rate of ^{137}Cs from fish is not related to metabolic rate (resting and active) is the variety of fish and their habitat from which the elimination data were derived (Rowan and Rasmussen 1995). These include carp (*Cyprinus carpio*), white crappie (*Pomoxis annularis*), bluegill (*Lepomis macrochirus*), summer flounder (*Paralichthys dentatus*), and brown trout (*Salmo trutta*) held in aquaria, large tanks, and small ponds, as well as wild arctic char (*Salvelinus malma*), brown trout, and northern pike (*Esox lucius*). These data cover a wide range in body size (0.1–100 g), life-history stage (larval, immature, mature), and activity. For example, flounder and northern pike are not very active in comparison with brown trout. The brown trout data (Udegal et al. 1992) also cover a size range of 12–416 g, including both immature and mature fish. The bluegill data (Kolehmainen 1972) also include immature and mature fish, ranging in size from 0.5 to 120 g. Yet in spite of all of the differences between these fish, they all fit the same line with regard to temperature and body size, with an uncertainty in the prediction of ^{137}Cs elimination of about 10%. If elimination rates were directly related to metabolic rates and especially activity, then we would have expected significant residual effects, which we did not find.

In addition, if the elimination of ^{137}Cs was directly related to metabolic rates, then the exponents for the weight terms in ^{137}Cs elimination and respiration models should be identical. However, the weight exponent in the elimination model is only –0.11 (Rowan and Rasmussen 1995), two- to three-fold lower than those found for respiration in most fish (Hewett and Johnson 1992). Almost any fish physiological process can be approximated by a function of body size and temperature, making correlations between unrelated processes likely. We believe that ^{137}Cs elimination is passive, a simple diffusion from a concentrated compartment (fish) to a dilute compartment (water). This hypothesis would explain the weak weight dependence and would make more sense for a nonessential isotope that occurs at ultratrace levels in fish ($<10^{-6}$ ppt). Of course, well-constructed laboratory experiments are needed to test these hypotheses. However, even if there is a link between metabolic costs and ^{137}Cs elimination, it only means that our activity estimates are the minimum, with true values even higher.

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Dr. Colin R. Bell
Microbial Ecology Laboratory
Department of Biology
Acadia University
Wolfville, Nova Scotia
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